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A
MANUAL OF HISTOLOGY
AND OF
HISTOLOGICAL METHODS.

BY

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PREFACE.

THIS little book has been put together with the view of supplying a want which I have frequently heard expressed by students—the want, namely, of a volume which should contain a concise but connected description of the minute structure of the tissues and organs of the body, and at the same time should give directions for their examination by the microscope.

The descriptive part is printed in large type; the practical exercises, which are placed at the end of each chapter, in smaller characters. In the Appendix some methods are described which could not have been conveniently introduced into the body of the work.

The whole represents the Course of Practical Histology which is given each summer in Trinity College.

I have decided to publish the book without drawings, because it is impossible to get original microscopic drawings made in Dublin, and I was unwilling to add to the expense and bulk of the volume by reproducing in its pages the figures of other authors, more particularly as our students have, in Quain's 'Anatomy,' a histological atlas which could scarcely be surpassed. Besides, I was writing primarily for students working in a laboratory, who always have access to drawings, diagrams, and preparations. I have found that the imagination of students is assisted much more by diagrams drawn in coloured chalks on a black board than by finished and accurate drawings of actual preparations. My original intention was to reproduce the diagrams which I draw each year to illustrate my Lectures and Demonstrations, but the great expense of printing in colours obliged me to give up this idea.

The book, written as it is for beginners, makes no pretension to originality. It will be seen on every page how deeply indebted I am to the works of Klein, Krause, Ranvier, Schwalbe, and other writers. I have entered into no discussion on doubtful questions, but have, in each case, given that view which seems to me most probable at the present time.

I have to express my sincere gratitude to the Provost and Senior Fellows of Trinity College for their liberality in undertaking the cost of publication.

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PHYSIOLOGICAL LABORATORY,
TRINITY COLLEGE, DUBLIN,
January, 1884.

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ERRATA.

Page 43, line 9 from top, *for* lymphatic *read* lymphatics.
Page 89, line 10 from bottom, *for* bones *read* bone.
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CHAPTER I.

INTRODUCTION.

Modern microscopes present an almost infinite variety in the details of their construction, but every instrument possesses the following parts, which are essential:—

From a solid and steady basis or **foot**, on which the whole instrument rests, there arises an upright piece of metal, to which are attached, in succession from below upwards, firstly, a concave **mirror**, so fixed that it can be inclined at any angle, and placed in different positions.

Secondly, a shelf, the **stage**, perforated in the centre by a round opening, whose diameter can be made to vary either by inserting into it plugs pierced by smaller holes of different sizes, or by rotating below it a perforated plate of metal called a diaphragm.

Thirdly, an arm, which carries a brass collar, in which slides the **tube** of the microscope.

In many microscopes the upright to which these parts are attached is fixed in a vertical position; the stage is then horizontal, and the tube vertical; but in other instruments the upright is capable of inclination, when of course the tube and the stage will also become inclined, but will always retain their relative positions, so that the axis of the tube is always perpendicular to the plane of the stage, with the centre of the perforation in which it coincides.

The tube usually consists of two pieces, which slide on one another as do the tubes of a telescope, and by pushing these pieces together, or drawing them out, the tube can be shortened or elongated.

Into the upper end of the tube fits a system of two lenses, which together form the **eye-piece.** On the lower end is a screw, by which there can be attached to the tube systems of lenses, called **object-glasses**, or **objectives**, of which each microscope must possess at least two.

The object-glass and the eye-piece are the essential optical parts of a microscope, and they act so that the object-glass forms an enlarged and inverted image of the object in the eye-piece, by the upper lens of which this image is still further magnified, as any object is by an ordinary pocket lens.

The image must always be formed for each eye-piece at a certain distance below the latter; and in order that this shall be the case, an arrangement has to be provided by which, according to the different object-glasses used, and to the varying length of the tube, the distance between the object and the object-glass can be made to vary.

The object lies on the stage, and is fixed in its position. Hence the tube carrying the object-glass must be movable, so as to be approximated to or withdrawn from the object. This movement of the tube has to be effected with great accuracy, and is called focussing the microscope for the object, or bringing the object into focus. It is carried out in two stages. First, the distance required is approximately got by sliding the tube up or down in the brass collar which holds it. This is called the **coarse adjustment**, and is carried out by the hand. In more expensive microscopes it is effected by a rack and pinion. By these means the necessary accuracy of adjustment could never be attained. This **fine adjustment** is arrived at by means of a screw, which is placed at the top of the upright, which carries the mirror, stage, and tube. This screw is provided with a milled head, which moves in a horizontal plane, and, when turned, causes the tube to move to or from the stage, but very slowly, so that the motion can be stopped as soon as the right distance is attained, and the object is seen clearly.

When the screw of the fine adjustment is turned from left to right, as if it were being screwed into the brass of the microscope, the tube is moved downwards; when in the opposite direction, the tube is raised. The range of motion which can be given to the tube by the fine adjustment is not very great, and the screw should always be kept about the middle of the range. If it be screwed down as far as it will go, it should be unscrewed half way, and, if necessary, the tube lowered by the coarse adjustment. If it be at its upper limit, the tube should be raised by the coarse adjustment, and the screw then brought to the middle of its range. The screw should always be worked with a light hand, and no attempt should be made to force it beyond its due limits of movement, lest the thread be injured.

The object-glasses give images of different sizes, and are commonly spoken of as **high** and **low powers**. Each consists of several pieces screwed together, and great care should be taken, when removing an object-glass from the tube, that the whole of it is taken off, and that it is not screwed asunder, leaving part of it attached to the tube. The parts of an object-glass should never be separated from one another.

English object-glasses are called by inches, or fractions of an inch: thus we speak of an inch, a quarter, an eighth, &c. These are the **focal lengths**, but the actual distance between the lower end of the object-glass and the object, when the latter is in focus, is always much less than the focal length. This distance is what is called the **working distance**, and with the higher powers is very short indeed.

Foreign makers designate their objectives by numbers or letters, which are arbitrary, each maker having his own series of glasses.

A student's microscope should have a half-inch and a fifth or sixth, and a two-inch will be found very useful for many purposes. No beginner should attempt to use very high powers. The difficulties of microscopy increase enormously the higher the powers which are used.

In using the lower powers there can be no risk of going wrong, but when the higher powers are employed the greatest care is necessary, in consequence of the very short interval between the object and the object-glass. The latter may get smeared with fluid from the object, or, if the object-glass be brought down too far, the object will be crushed or broken. Before placing the object on the slide, or removing it, the tube of the microscope should always be raised. This will at first cause a little trouble, as the process of focussing will have to be gone through again ; but it will save time in the long run, by ensuring both the objects and the object-glass from injury. If fluid get on the object-glass, this should be wiped carefully with a soft cloth or piece of chamois leather. But this is a process which should be required only very rarely. The greatest possible care should be taken to preserve the object-glasses from falls or other mechanical violence.

Most microscopes have two or more eye-pieces, some of which magnify the image formed by the objective more highly than others. In this way, with the same objective, different degrees of amplification can be got. In general, it is not well to increase the power of the microscope by the eye-piece, for any defects in the image are in this way magnified, and unless the objective be very good, what is gained in amplification is lost in distinctness.

With the same objective and eye-piece, the longer the tube of the microscope is made the greater is the amplification of the image, and the higher the magnifying power.

An excellent student's microscope can be had for from about four to six guineas.

Objects are examined with the microscope in two ways—either by **transmitted** or by **reflected light.** For the first method the object must be transparent. It lies on the stage over the perforation; the light of a lamp or of the sun is caught on the mirror, and made to traverse the object, and thus pass through the object-glass and the eyepiece, and so reach the eye of the observer, This method is employed almost without exception in histological work.

By the other method the hole in the stage is closed; the light is concentrated on the object from above by means of a large lens, called a **condenser**, and the light reflected from the object passes through the tube of the microscope to the eye. By this method the object is viewed as we view ordinary objects about us, while in viewing an object by transmitted light, we examine it as we do the images on a stained glass window. The best light for ordinary microscopic work is that reflected from a white cloud.

Objects to be examined are placed on a piece of glass called a **slide.** This is usually three inches long by one broad. Slides should be kept scrupulously clean.

The object is almost always immersed in some fluid, and is covered by a small square or circular piece of very thin glass, which is called a **cover-glass.** Great care must be taken in cleaning cover-glasses, as they are expensive and very easily broken. New cover-glasses are sometimes difficult to clean. If this is the case, they should be placed in oil of vitriol for some time, and then washed in water and dried with a soft cloth. Cover-glasses, after being cleaned, should never be touched, except on the edges. The contact of even the cleanest finger with the surface of the glass is sure to leave a stain on it. Before commencing to make a preparation, slides, cover-glasses, and everything else requisite, should be clean and ready at hand.

The following **apparatus** will be required:—Slides, cover-glasses, watch-glasses, a glass plate three inches square, two needles set in handles, a camel's-hair brush, a piece of glass rod, a pair of fine forceps, a pair of scissors, a small scalpel, a razor, a section-lifter, some pieces of filter-paper, reagents.

For **preliminary exercise** in the use of the microscope some simple objects should be mounted and examined.

1. Screw on the low power objective. Bring down the tube of the microscope until the lower end of the object-glass is about an inch from the stage. Arrange the diaphragm so that there may be a large hole in the stage. Look into the microscope while the mirror is moved slightly from side to side until the field of the microscope appears as an evenly-lighted circle. The mirror must be kept, whatever its inclination may be, in such a position that the centre is vertically below the centre of the aperture in the stage, and in the line of the axis of the tube of the microscope. If it be swung from this position the light will not pass straight through the object to the eye, but will fall obliquely across the object, which will then be seen in great part by light diffused from its surface. Such an arrangement of the mirror is said to give '**oblique illumination**,' which is very useful for certain kinds of objects, but is rarely required in histological work, in which the illumination should always be '**central**.'

Some black spots will possibly be seen in the clearly-illuminated field. These are probably specks of dust on one of the glasses of the eye-piece. If so, they will turn as the eye-piece is rotated in the tube. They will be probably found on the lower surface of the eye-piece, from which they can be easily wiped off. Spots of dust on the eye-piece appear as spots; but if they are on the object-glass, they show themselves by a general cloudiness and want of distinctness in the object under examination.

Having arranged the microscope, get ready a slide and cover-glass, and have at hand a saucer with some clean water.

Scrape the cut surface of a raw potato gently with a knife, and place in the centre of the slide a small drop of the turbid fluid thus obtained. Place near to it with a glass rod a drop of water of about the same size, and mix the two drops together with the rod or with the point of a needle. Then take the cover-glass, holding it by the edges, and bring it down until it just touches the convexity of the drop of fluid, and then let it fall by its own weight on the slide. In this way it will spread out the fluid in a thin layer, and not include any air-bubbles. Or one edge of the cover-glass may be allowed to rest on the slide beside the drop of fluid, while the opposite edge is supported by a needle. The needle is gradually approached to the slide and finally withdrawn. In this way the cover-glass rotates on its first-mentioned edge as on a hinge.

If now it is found that the quantity of fluid between the two glasses is so great that the cover-glass floats and readily moves about on the slide, a small piece of filter-paper is applied at one edge of the cover-glass, and allowed to absorb the fluid until the cover-glass adheres slightly to the slide. On the other hand, if the drop be not large enough to fill the space between the glasses, and if part of this be occupied by air, a small drop of water may be applied at the edge of the cover-glass, and allowed to run in by capillarity until the space is full, when the excess may be removed with filter-paper. In all cases the amount of fluid should be sufficient to completely fill the space between the slide and cover-glass, so as to leave no room for air; but the layer of fluid should be of extreme thinness, so that the objects contained in it may not lie one over the other, and obscure the view.

Place the slide on the stage of the microscope, the object lying over the aperture in the stage, and, while looking into the instrument, bring the tube down by a very gradual screwing movement, and so make the coarse adjustment. When a certain point is reached the object will be seen, but still misty and badly defined. Then it is best to abandon the movement by the hand, and to complete the focussing, or to make the fine adjustment by the micrometer screw. This is very gently turned until the objects in the field take shape and appear with well-defined outlines. When at this point, the screw is moved in one and the other direction, to make sure that the exactly right focus has been attained.

In the present case numerous little particles will be seen, differing a good deal in size, having a more or less oval shape, and appearing with a bright centre and a dark margin. These are **starch granules**, of which the bulk of the potato is formed.

There will possibly be seen other bodies of a round, or altogether irregular shape, and which may be of any size, but are generally much larger than the starch grains. They have a broad black margin. These are **air bubbles**. The cleaner and freer from grease the slides and cover-glasses are kept, the fewer will be the air bubbles.

2. Leave the object on the stage; withdraw the tube, and replace the half-inch by the high power, one-fifth or one-sixth. Focus as before, first moving down the tube with the hand

until something is visible, and then completing the adjustment with the screw. Great care must be used in focussing with the high power, as the working distance is very short.

The starch grains will now be seen as much larger objects, and on each of the larger grains there will be noticed a spot with a series of fine lines arranged concentrically about it. This shows that the starch grain is not homogeneous, but has a well-defined laminated structure.

Select a starch grain of small size, and move the fine adjustment until the black line bounding the object is as narrow as possible. Then alter the focus so as slightly to raise the tube, and thus throw the object somewhat out of focus by making the distance too great. The centre of the granule will become brighter than before, and the margin broader and darker. This is due to the fact that the granule has a convex surface, and is of a higher refractive power than the water in which it is immersed. It consequently acts as a convex lens, and brings the light to a focus above it. When we focus for the object itself, we see the base of the cone of light; but when we focus for a higher point we see a section of a narrow part of it. All the light passing through the object is brought into the centre; hence this is bright, and the margin, from which the light is bent aside, is dark. This behaviour as regards the light is common to all objects whose refractive power is higher than the medium in which they are placed, and whose surfaces are convex.

Try to find an air bubble—if possible a small one. Whatever the position of the tube, the margin will be much darker and broader than that of the starch grain. Alter slightly the focus, first in one, then in the other direction. On raising the tube, the whole bubble will become indistinct; but on lowering the tube, so as to make the distance between the object-glass and the object too small, the centre of the bubble will become brighter, and the margin broader and darker. This depends on the circumstance that air has a lower refractive power than water. The black margin is due to the total reflection of those rays of light which strike the lower convex surface of the bubble very obliquely, while those rays which strike the bubble less obliquely and pass through, are not made to converge above, but to diverge. Consequently, by focussing down, we get a smaller but a brighter circle of light, that is, the point before the divergence of the rays has taken place; while above the light is less intense, because the rays have diverged, and the same quantity of light is spread over a larger surface.

All bodies with convex surfaces, and which are of a lower refractive power than the medium in which they are placed, will behave as the air bubble. Bodies having concave surfaces, although they may be of higher refractive power than the fluid in which they are placed, will cause the light to diverge, and will hence behave, on altering the focus, like an air bubble.

These facts are of great importance, and should be continually borne in mind.

The dark margin of an air bubble is frequently divided into two or more concentric bands by fine bright circles, which are due to diffraction of the light by the edge of the bubble.

3. To the same object which served for the two preceding observations a reagent may be now applied. The method by which reagents are applied to objects under the microscope, or by which one fluid is replaced by another, without disturbing the object-glass, is what is called the **'method of irrigation.'**

A small drop of a very weak solution of iodine is placed on the slide, so as to touch one edge of the cover-glass, but on no account to flow over its surface. The iodine will immediately begin to run in between the glasses, and on looking into the microscope the granules will be seen all moving across the field, apparently *towards* the side on which the drop was placed. Their motion, however, is really in the opposite direction, since everything appears inverted when seen in the microscope. If the granules are watched attentively, many of them will be seen to roll over and over in the current, and they will be seen in this way from opposite sides, and their shape may be determined. They will appear of pretty much the same shape in all positions, and we hence conclude that they are oval like an egg, and not like an oval cut out of a piece of paper, which would present a very different appearance according as it was seen from the edge or from the surface. It is by making microscopic objects roll that their shape is judged of. This method is of general application.

When the iodine comes into contact with the starch grain, this will instantly assume a blue or black colour, according to the strength of the reagent.

If the iodine were allowed to run under the cover-glass, and nothing else done, we should soon have too much fluid between the glasses, and the water would be merely mixed with, not replaced by, the iodine; a piece of filter-paper therefore is placed on the slide touching the opposite edge of the cover-glass to that at which the iodine ran in, and thus the water is sucked out at one side, while the new fluid runs in at the other. In this way a rapid current is caused between the glasses. Many of the granules will be swept away in this current, but sufficient will remain to show the reaction. When this is complete the current can be stopped by taking away the filter-paper. A preparation can be 'irrigated' for any length of time by this method, remaining under observation during the process. It is only necessary to renew the drop of the reagent at one side according as it is exhausted, and replace by fresh pieces the filter-paper at the other as each piece becomes soaked.

4. Pull up the tube; remove the slide, and clean it and the

cover-glass. Place on the slide a small drop of milk; cover, and remove any excess of fluid, as already directed. Examine with the high power. A large number of minute round bodies cover the field; they differ in size, but are all smaller than the starch grains. As they roll over they appear always round; hence they are spherical. On altering the focus they behave like starch granules; when the tube is slightly raised the centre becomes brighter, the margin broader and darker; hence they are more highly refractive than the fluid in which they float. These bodies are **fat globules.** It is to the light reflected from these that the milk owes its white colour and opacity. The clear colourless fluid in which they float is invisible in the microscope.

5. Clean the slide, and place on it a few threads of **cotton wool.** Put, with a glass rod, a drop of water on the cover-glass. Invert this, and bring it down on the threads. If there is excess of water, remove it with filter-paper. Examine with the high power. Each thread appears as a colourless band, which is every here and there twisted on its axis. Where the twist occurs it can be seen that the thread is flattened, since it presents a narrow edge as it turns over.

Irrigate with a strong solution of iodine, and then with water, so as to wash away the excess of the reagent. The threads assume a dark mahogany-brown colour. If they are then irrigated with strong sulphuric acid, each thread swells very much, and becomes of a beautiful blue colour. This is the reaction of **cellulose**, of which substance cotton threads are composed.

6. The appearance which an object presents depends very largely on the medium in which it is examined. If the refractive power of the medium be the same as that of the object, the latter will be altogether invisible, unless its colour differ from that of the medium; and the nearer the refractive power of the medium is to that of the object the more transparent and faint will the latter appear. On the other hand, if the difference in the refractive power of the medium and that of the object be very great, so much light will be reflected from the surfaces of contact of the medium with the object that the latter will appear too dark and opaque for examination by transmitted light.

It is necessary, therefore, always to take account of the refractive power of an object to be examined in choosing the medium in which the examination is to be made.

Most of the animal tissues have a very high refractive power, and to this they mainly owe their great opacity. Hence, in order to make them sufficiently transparent for microscopic examination, it is necessary to immerse them in fluids whose refractive power is high also, such as glycerine and Canada balsam, the media in which the greater number of histological objects are examined. It will be found that objects when in Canada balsam are much more transparent than when in glycerine. It is therefore necessary to stain objects which are to be mounted in balsam, in order to bring out the finer details of their structure, which would otherwise be lost by excess of transparency.

Get ready four slides and four cover-glasses. Place on each slide a very small quantity of finely-powdered starch, such as is used in the laundry. 1. Put the cover-glass on one preparation, without adding any fluid. 2. Put up the second in water; place a drop of water on the surface of the cover-glass; invert this so that the drop may be undermost; then gently apply the cover-glass to the object. 3. Put up the third in glycerine; and, 4. the fourth in oil of cloves, applying these fluids in the same way as was directed for the water in 2. Examine the four preparations in succession with the high power. The starch grains here differ from those of the potato. They are smaller, and are of a polygonal shape, presenting well-defined angles and facets. In 1 they appear as black spots, with bright centres, but in consequence of the opacity of their edges, their shape is unrecognizable. 2 and 3 show the shape of the granules very well; but in 3 they are evidently more transparent than in 2. In 4 the grains are so transparent that there is some difficulty in seeing them at all, and their outlines are so indistinct that their shape cannot be made out. This series of preparations illustrates the effect on the appearance of an object of a progressive increase in the refractive power of the medium in which it is examined. Air has the lowest, and oil of cloves the highest, refractive power.

CHAPTER II.

THE CELL.

Comparatively few tissues occur in the body. The numerous organs are formed of different arrangements and combinations of these tissues. In every tissue, elements are to be met with which are called **cells**, and whose importance, both anatomically and physiologically, is primary. The entire body arises from a single cell, the ovum, and up to a certain stage in development the entire body consists of a mass of cells which have arisen by repeated division of the ovum. At a later period, while some parts retain their purely cellular structure, in others there are formed between the cells, and doubtless through their agency, substances which differ in quantity and quality in different parts, and which by their differences determine the characters of the different tissues. These substances, as they lie between the cells, are called **inter-cellular substances**. In the earlier periods of life the cells are generally larger and more numerous in proportion to the intercellular substance than they are at a later period; and this seems to depend on their playing a prominent part in the growth and active nutritive processes which take place in youth.

A cell in its simplest form consists of a mass of a colourless, soft, granular matter, which is called **protoplasm**, in the interior of which is contained a generally round or oval body called a **nucleus**, which differs both in structure and in chemical constitution from the protoplasm. The **granules** which occur in protoplasm are not all of the same kind: some of them are large and easily to be seen as separate points; others are of extreme fineness; the protoplasm, according as it contains granules of one or the other kind, is said to be coarsely or finely granular. The granules are frequently irregularly distributed, being more abundant at one part of the cell than at another. In their chemical constitution the granules of cells differ greatly.

Whether protoplasm is structureless and homogeneous, or possessed of different parts, is uncertain. Some observers maintain that the substance of every cell consists of at least two parts, one existing in the shape of a fine network, the other lying in the meshes of this. This view is not, however, universally accepted. Protoplasm possesses the power of spontaneous movement, which shows itself not only in changes in the shape of the cell, but in its passage from place to place. In most of the tissues of the body, cells are to be found, which are not fixed in their position, but creep about by virtue of their powers of movement, and are hence called wandering or migrating cells, and their movements are called amœboid movements.

Protoplasm has the power of taking into its interior solid substances foreign to itself. It either stretches out processes by which it seizes the foreign body, and draws this into its interior, or it spreads itself over and around its prey. The substance absorbed may, according to its nature, undergo a total or partial solution or digestion, or may be ejected unchanged.

A cell composed of a mass of protoplasm such as has been described, has, of course, no fixed shape; but most of the cells in the bodies of animals have very constant and definite forms. These cells have lost their powers of spontaneous movement, and have often undergone a peculiar change by which a layer on the surface has come to differ from the protoplasm in the interior, both chemically, and also in consistence, being firmer, harder, and more unyielding. This differentiated layer is what is called a **cell**

wall. The various shapes which cells assume in becoming fixed in the tissues are to be explained on mechanical principles, growth taking place in the direction of least resistance, and being impeded in those directions in which the resistance is great.

Many cells have the property of forming matters which they store up in their interior. In vegetables we find cells filled with chlorophyll, or the green colouring matter of plants, and the largest part of our food comes from the starch which is formed and stored in vegetable cells. In the animal body we find a similar formation and accumulation in cells of pigment, fat, and other matters. An analogous process occurs in all the gland cells, only here the accumulation is temporary; the materials of the various secretions are formed by the cells during the rest of the gland, and discharged when the secretion takes place. Many of these materials for secretion appear in the gland-cells in the shape of granules.

Not only may the outer layer of a cell become transformed, but the entire mass of protoplasm may undergo transformation, when it naturally loses its protoplasmic or vital properties; and although it may continue for a time to form part of the body, it must be looked on in a certain sense as dead matter. The transformation may extend not only to the protoplasm, but to the nucleus also, or the nucleus may be spared, although it can scarcely escape alteration. As long, however, as a small residue of untransformed protoplasm remains, this may, under favourable circumstances, again increase in quantity and regain all its vital endowments.

Protoplasm never arises from dead matter, but always from pre-existent protoplasm.

In accordance with this law, cells never arise from formless or dead matter, but every cell is the descendant of a parent from which it arises by a process of division.

The **division of a cell** is preceded by certain changes in its nucleus, which itself divides prior to the division of the protoplasm: as these changes are manifestly of an active kind, the process which goes on in the nucleus is called **karyokinesis.**

Under ordinary circumstances, when a cell is at rest its nucleus is a vesicular body enclosed by a distinct membrane. Its consistence is fluid or semifluid, and it presents a network attached to the membrane, and traversing the whole nucleus. In the meshes of this network the fluid material is contained. The nucleus differs chemically from the cell substance; for while this swells and becomes pale with acetic acid, the nucleus shrinks and increases in distinctness. Hence acetic acid is commonly employed to demonstrate or "bring out" nuclei. Furthermore, many staining fluids which do not affect the substance of the cell fasten on the nucleus. These stain the nuclear network most, but also the inter-reticular fluid.

At certain points in the nuclear network thickenings occur which are called **nucleoli.**

When the cell is about to divide, the nucleus undergoes the following alterations:—The nuclear membrane becomes indistinct or vanishes, but still the outline of the enlarged nucleus is evident as a clear border. The network becomes much closer than it was in the resting state, and stains much more deeply, while the substance contained in its meshes stains less or not at all. The nucleoli disappear.

In the further stages, the network, still staining deeply, changes its form, and assumes in succession the appearance of a convoluted thread, of a wreath-like figure, of a star whose centre is at that of the nucleus and whose rays diverge towards the periphery, and of a compressed, flattened figure, sometimes continued at both sides into a spindle by threads which stain only feebly. At this stage the nucleus is oval, and the compressed figure is extended across it at right angles to its long axis, and is for this reason called the equatorial plate. This divides in its middle, that is in the equator of the nucleus, and the two portions, retreating one from the other, assumes each a stellate shape. There are then two daughter nuclei, arisen by division from the old parent nucleus. These daughter nuclei, which are at first smaller than the parent, pass from the stellate form through that of the convolution back to the condition of the resting nucleus. Shortly after the separation of the daughter nuclei the cell-substance becomes constricted between them and ultimately divides, one nucleus being contained in each division. These changes in the nucleus, preceding and accompanying the division of cells, are, it would appear, common to all classes of animals and to vegetables. Their discovery is of great importance, since the existence in a part of the peculiar nuclear figures is proof that the cells there are multiplying—a proof which was hitherto wanting, the division of cells having been in most cases only a matter of inference, and sometimes exceedingly uncertain.

It is held by some observers that a division of nuclei can take place without the occurrence of the changes just described. In this case the resting nucleus, without altering its ordinary structure, simply becomes constricted and divides, its division being followed by that of the cell. This is called **direct division,** while that by karyokinesis is called **indirect**.

It is probable that there are other modes by which the division of nuclei is effected. One of these has recently been described under the term **fragmentation**. By it small portions of the nucleus are, as it were, budded off, and the pieces about to become separate are frequently for a time connected by a pedicle with the parent nucleus. The division may be preceded by karyokinetic changes in the nuclear network, or may be direct. It has been as yet studied only in the cells of the red marrow of the bones (J. Arnold).

We know that no cell arises except by descent from a pre-existent cell. But whether or not a nucleus ever arises in the interior of a cell, except by division of a parent nucleus, is a question which cannot yet be certainly answered, although the evidence inclines more and more in favour of extending the aphorism *omnis cellula e cellula* to *omnis nucleus e nucleo*.

As has been stated, there are few parts of the body which retain an exclusively cellular structure; but in most places there is formed between the cells more or less of an intercellular substance whose structure and disposition very largely determine the shape and arrangement which the cells themselves present in the mature tissues.

What part is played by the cells in the formation of the intercellular substances is exceedingly obscure.

According to some, the intercellular substance is formed from the protoplasm of the cells, the transformation affecting only the outer part of some cells, but involving the whole of others. Other writers look on the formation of the intercellular substance as due to a process similar to that of secretion carried on by the cells, which, while not themselves undergoing change, produce in their interior matters which are subsequently separated and organised; while a third school seems inclined to deny to the cells any direct share in the production of the intercellular substances. It is quite possible that the process is not the same in all the tissues.

CHAPTER III.

BLOOD.

Blood, when circulating in the vessels, or when recently withdrawn, appears to be a homogeneous fluid; but examination even with the unaided eye shows it to differ from an ordinary solution, since it is opaque even in very thin layers.

When examined by the microscope it loses its apparent homogeneity, and is seen to consist of a clear colourless fluid (liquor sanguinis or plasma), in which float a vast number of little particles (corpuscles).

The **liquor sanguinis** is at first invisible, since it is perfectly structureless and without colour; but after a time there comes to be formed in it a substance which is solid, and in microscopic preparations takes the form of an exceedingly fine network. This is **fibrin**.

The **corpuscles** are, in the blood of all vertebrate animals, evidently of two kinds, and are distinguished by the fact that one kind are colourless (white corpuscles), while the other kind are of a faint yellow colour (red corpuscles). These latter are much more numerous than the white corpuscles, and it is to them that the colour of the blood is entirely due; for although when seen singly they are pale yellow, yet when occurring together in large numbers they appear red.

The **red corpuscles** are of two kinds. In mammalian animals they have no nuclei, while in the oviparous vertebrates they are nucleated. Nucleated red blood corpuscles occur in mammals during the early stage of embryonic life, and occasionally, in small number, in disease.

In ovipara the red corpuscles are flat oval bodies, varying considerably in size in different animals, but being always larger than the corpuscles of mammalian blood. In the middle of each corpuscle there is an oval nucleus, which slightly bulges the sides, and between this and the periphery the colouring matter is contained. The corpuscle is composed of a soft, flexible, highly elastic substance, which seems to become somewhat more dense and rigid towards the surface, but there is no definite and separable cell-wall. The nucleus is generally not seen at first, but after the preparation has been made for some little time it gradually becomes apparent, and can always be readily demonstrated by the application of reagents. It has a granular appearance, and in favourable circumstances can be seen to contain a beautiful reticulum. The corpuscles frequently show, particularly if the preparation has been submitted to violence, radial markings due to folds passing from the nucleus to the periphery. That these markings are so caused can be readily determined by the use of the fine adjustment.

In mammals the red blood corpuscles are, except in a few animals, circular flattened bodies, slightly concave on their surfaces, and rounded at the edge. Camels and llamas are exceptional, in that their corpuscles are flattened ovals, and of extremely small size. In no case does a nucleus exist. Neither in mammals nor in ovipara does any proportion exist between the size of the animal and that of his red corpuscles. The human red corpuscle is, as compared with other mammalian corpuscles, a large one. Its diameter is on an average $\frac{1}{3200}$ inch ; its thickness $\frac{1}{12400}$ inch. These corpuscles are uniformly coloured. They possess the same flexibility and elasticity as do the nucleated corpuscles of ovipara, and seem to have no cell-wall. After withdrawal from the body, and even in the vessels when the circulation is slow, they manifest a remarkable tendency to adhere to one another by their surfaces, so as to form continuous masses, like piles of coin. Numerous attempts have been made to explain this curious phenomenon, but none are satisfactory. It is manifestly dependent on, or at all events connected with, the normal biconcave shape of the disc, for all circumstances which alter this shape prevent the formation of the rouleaux. The corpuscles of ovipara do not adhere in rouleaux, possibly in consequence of the bulging of the sides by the nuclei.

The **colourless or white corpuscles** in the different classes of animals differ less fundamentally than do the red. They are always true cells, as already explained, consisting of a mass of protoplasm surrounding one or more nuclei, and capable of performing amœboid movements, of taking up solid particles into their interior, and of multiplying by division, none of which properties are enjoyed by the red corpuscles. When the white corpuscles are at rest, or dead, they assume a spherical shape. The size of the colourless corpuscles does not vary in the different members of the animal series to the extent to which the red corpuscles do, while in the same animal there is greater difference of size among the white than among the red corpuscles. In ovipara they are usually smaller than the red; in mammals they are larger. In man the diameter varies from $\frac{1}{2400}$ to $\frac{1}{1000}$ inch. The nucleus is usually not visible until the cell has died, but it can readily be made manifest by reagents. It is sometimes single and round, sometimes irregular in shape, and not uncommonly multiple. The protoplasm surrounding the nucleus varies in amount, and thus determines the size of the corpuscle. In the protoplasm are embedded granules, which are sometimes exceedingly fine, at others very coarse. In the latter case the granules are usually not evenly distributed, but massed together at one place, and their position changes during the amœboid movements of the corpuscle. The colourless corpuscles have no cell-wall.

Besides the red and colourless corpuscles, which have been long known, **other formed elements** have been recently described as existing in the blood. These bodies in mammalian blood are discoid, non-nucleated, and colourless, or very faintly coloured. As their refractive power is almost the same as that of the liquor sanguinis, they are exceedingly transparent objects, and in some cases quite invisible, and recognizable only as round spaces, about which the other corpuscles are crowded together. They break down very rapidly after the withdrawal of the blood, and would appear to take an important part in the formation of fibrin. They are probably red corpuscles in an immature condition. They have been particularly described by Dr. Norris of Birmingham, and by Bizzozero; but there are differences in the descriptions which make it doubtful whether these writers refer to the same bodies. Much doubt has been thrown on the existence of the corpuscles described by Norris, and they are believed by some observers to be only ordinary red corpuscles, which have been caused to discharge their colouring matter by the methods employed in making the preparations. Bizzozero professes to have seen his corpuscles, which are much smaller than those described by Norris, while the blood was still circulating in the vessels of the living animal. Analogous colourless bodies, but nucleated, exist in the blood of ovipara.

A few minutes after a preparation of blood has been made it will be seen, on raising the cover-glass, that the drop has solidified into a soft gelatinous film. This is due to the formation in the liquor sanguinis of a very abundant, but extremely fine network of colourless transparent threads, in the meshes of which the corpuscles lie. As these greatly obscure the network, the latter is better seen after the removal of the corpuscles by washing.

There will then be noticed at the nodal points of the network little granular masses, which are probably the debris of the colourless bodies described above.

By the application of reagents many important facts relative to the structure of the blood corpuscles may be demonstrated.

If a drop of blood be mixed with an equal bulk of water it is seen to lose its opacity, and at the same time to become darker in colour when viewed by reflected light. The blood is now said to be "**laky**," a condition which can be brought about by various other agencies besides water. If the water be made to act slowly, and the stages of its action watched under the microscope, the following will be seen:—In blood of ovipara the red corpuscles alter their shape, and become spherical. The nuclei become very apparent, and frequently occupy an excentric position in the corpuscles. The colouring matter rapidly leaves the corpuscles, and diffuses out into the surrounding fluid, so that at a certain stage we have colourless bodies floating in a red or yellow fluid. The outlines of the corpuscles become more and more faint, and finally the nucleus only is visible as an oval granular body. The white corpuscles cease their amœboid movements, become spherical, their nuclei become evident, and their granules often exhibit a peculiar dancing or vibratory movement in the interior of the cell.

In mammalian blood also, on the application of water, the red corpuscles alter in shape, which causes a breaking up of rouleaux, if they existed. The biconcave disc becomes concavo-convex, the opening of the concavity narrows, and the margins finally coalesce, a spherical body resulting. This gives out its colouring matter, and remains visible for a time in the stained fluid, but becomes gradually more faint, and finally disappears. The white corpuscles behave as in the blood of ovipara.

This important experiment shows that the red corpuscle consists of two parts, which can be separated one from the other, namely, the colouring matter, or **hæmoglobin**, and a colourless **stroma**, with which under ordinary circumstances this hæmoglobin is in some way combined. It also shows that the opacity and bright colour of the blood are due to the red corpuscles, which reflect most of the light which falls on them; but when their colouring matter is removed, the refractive power of the stroma is so reduced as to allow the light to pass freely through the blood, instead of undergoing reflection. The blood becomes in consequence transparent, and of a dark colour when viewed by reflected light.

An effect on the blood similar to that caused by water can be produced by the action of dilute acids, or alkalis, by ether, chloroform, by passing continuous or interrupted electric currents through the blood, by repeated freezing and thawing, and by many other means. In all these cases the colouring matter passes into solution, and the corpuscles are either dissolved, or remain as faint colourless bodies, while the blood assumes the dark transparent appearance to which the term laky is applied.

If the blood be mixed with a neutral saline solution of greater density than the liquor sanguinis, as, for instance, a 10 per cent. solution of common salt, the corpuscles undergo an alteration of the opposite kind to that which they experience when water is added. The red corpuscles lose their regular shape and assume a crumpled appearance ; and in the blood of ovipara the radial plication already noticed is often very well marked. The corpuscles appear of a darker colour than before, their outlines harder and more defined, and their smooth homogeneous substance becomes more or less granular. In the blood of ovipara the nuclei become evident, while in vertebrate blood the rouleaux break up. The white corpuscles become spherical, and cease to move. To the naked eye the blood, when mixed with the saline solution, becomes much brighter in colour, and more opaque than before. This is due to the increased density of the corpuscles, which now reflect more and transmit less light than they did previously. We see from the action of water on the one hand, and of strong saline solution on the other, that the red corpuscles present the phenomena of osmosis; absorbing fluid, or giving it out according as the liquor sanguinis is unduly diluted or concentrated.

If a preparation of mammalian blood be not covered directly it is withdrawn, it is common to see a large number of the red corpuscles covered with spines, something like the outside of a horse-chestnut. A similar appearance is often noticed at the edge of preparations which have been made for some time. This prickly appearance is much more frequently observed in some specimens of blood than in others, and is peculiarly common in the blood of persons suffering from fever. Its explanation is not certainly known. It is not due to concentration of the plasma, since it is not common after addition of strong saline solution; while the fact that corpuscles so altered regain their smooth appearance when exposed to an atmosphere of carbonic acid would point to the loss of this gas as having some share in the production of the altered form. A spinous condition of the corpuscle does not occur in ovipara.

When mammalian blood is acted on by a solution of tannic acid the surface of the red corpuscle becomes coagulated, and gives way at one or more points; the fissure is very commonly of a crescentic form, and situated at a little distance from the edge. From the fissure a substance protrudes, which at the moment of its protrusion is coagulated, commonly as a little shining knob, but sometimes as a long projection. In these knobs or projections is contained the greater part of the colouring matter of the corpuscle, but the whole of the colouring matter usually does not pass into the protrusions, and these consist of some substance besides the colouring matter, for they stain readily in magenta, which does not stain hæmoglobin. Hence tannic acid does not, as sometimes stated, effect a separation of the colouring matter from the stroma, but brings about an analysis of some other kind, the exact nature of which is uncertain. Tannic acid produces analogous results in the blood of ovipara, but here the reaction does not succeed so constantly as in vertebrate blood.

¶ When the red corpuscle of the newt or (although less constantly) the frog are treated with a solution of boracic acid the colouring matter sometimes collects itself about the nucleus, leaving the peripheral parts of the corpuscle. At a certain stage in the process the colouring matter is still connected at points with the periphery of the corpuscle, and in this way assumes a stellate shape, which must not be confounded with the radial plication already referred to. Subsequently the nucleus and colouring matter are protruded from the corpuscle. Somewhat similar appearances are sometimes seen at certain stages in the action of water on the corpuscles.

When mammalian blood is heated to 52° C. the rouleaux break up, and the corpuscles undergo peculiar alterations of form, becoming at first deeply notched at their margins, then throwing out long beaded processes, or several small globular projections, and finally, after a time, breaking up into minute globular bodies, which retain their colour. If the heat be raised to 60° C. the blood becomes laky. Nucleated red corpuscles (frog's) at 45° C. become uneven on the surface, and notched or dumb-bell-shaped, but do not form the curious figures or break up into globular masses as do the mammalian corpuscles. These phenomena show that the red corpuscle does not consist of a cell-wall and fluid contents, but of an uniform soft substance which can break up into pieces. By mechanical means, as raising the cover-glass, or drawing some sharp instrument smartly across it, the blood corpuscles can often be divided, when it is seen that they do not evacuate their contents, but each fragment collects itself into a little globular mass.

Small spherical masses resembling those got by heat or mechanical violence are often found in the blood, even in health, but particularly in certain diseases (severe forms of anaemia). They are called **microcytes**. Their origin and import are uncertain.

Heat of 48° to 50° C. arrests the amœboid movements of the white corpuscles; but these may be resumed if the temperature be lowered. If, however, the high temperature be maintained, or still further raised, the cessation of motion is permanent. These phenomena are precisely analogous to heat tetanus and heat rigor of muscles.

While we must look on the white corpuscles as indifferent cells, there is much difficulty surrounding any attempt to explain the structure and nature of the red corpuscles. It is most probable that in ovipara they are cells which have retained their nuclei, but whose protoplasm is changed into the material of the stroma and hæmoglobin, in an analogous way to that in which, in the case of the epidermic cells, we shall see that the protoplasm is converted into keratin. In the corpuscular substance the hæmoglobin is combined with the stroma in some manner as yet imperfectly understood. The combination is a soft, flexible, elastic solid, without separable outer membrane, but very sensitive to changes in the density of the fluid in which it floats. It has lost all its protoplasmic properties, and is in a certain sense a dead substance. It has no power of spontaneous movement; it does not respond to stimuli; it cannot multiply by division; nor can it take up foreign particles into its interior. It is most probable that it cannot even nourish itself from the plasma; but that, having performed for a short time its oxygen-carrying function, which is inherent in the hæmoglobin, it undergoes destruction.

The mammalian corpuscle is most probably a cell which has lost its nucleus either by extrusion at an early stage of its formation or by atrophy, and whose protoplasm is transformed as above described. There are many reasons, both physiological and pathological, against the view that the corpuscle is a *nucleus* which has lost its protoplasmic surroundings and has acquired colour. This view has, however, recently been again advanced, and the question cannot, as yet, be considered as in any degree settled; the whole subject of the development of the blood being full of obscurity and difficulty.

The colouring matter of the blood, or hæmoglobin, presents certain interesting microscopical peculiarities. Although in chemical constitution one of the most complex substances known, yet it crystallizes, in some animals with great readiness (dog, rat, guinea-pig), in others with greater difficulty. The crystals differ in shape in different animals: in the dog they are long four-sided prisms; in the guinea-pig tetrahedra; in the hedgehog six-sided plates. Even in the same blood crystals of different form may be got; so in the same specimen of rat's blood we may find both prismatic-shaped crystals and hexagonal plates.

When blood has long been exposed to the air, or when it has been acted on by acids, alkalis, and other reagents, the hæmoglobin is decomposed, and gives rise to the substance which gives the brown colour to old blood stains. This substance is called **hæmatin**, and does not crystallize; but from it crystals can be obtained, whose presence is a valuable proof that the substance under examination is really blood. These crystals are formed of a compound of hæmatin and hydrochloric acid, called **hæmin.** They occur as minute rhombic crystals, of a dark-brown colour, and are frequently arranged in groups lying one across the other.

In old blood extravasations orange-coloured crystals are commonly met with, formed of a substance called **hæmatoidin.** This substance contains no iron, and is believed to be identical with bilirubin.

When a solution of hæmoglobin is examined with the spectroscope it is found to intercept certain rays of light, and to give rise to dark bands (absorption bands) in the spectrum between the Fraunhofer lines D and E. If the hæmoglobin be in the oxidised condition (oxyhæmoglobin) there are two bands; if in the deoxidised or reduced, only one, whose darkest part is midway between the two bands of oxyhæmoglobin. These spectra are a most valuable test for blood. By means of a spectroscope adapted to the microscope, or microspectroscope, the characteristic spectra can be obtained from extremely minute quantities of blood.

If the hæmoglobin have undergone decomposition by exposure or other cause, it will either give no spectrum at all, or one different from that of the unaltered colouring matter. The description, however, of these spectra belongs to the province of physiological chemistry.

The blood of the frog and that of man will serve as examples in the following exercises. The blood of other animals should, however, be examined as occasion offers:—

It is best to begin with the frog, since in it the red corpuscles are larger and fewer, and the distinction between the red and white corpuscles more manifest, than in human blood.

1. **Frog's Blood.**—A frog is killed by pithing or by chloroform. The sternum is raised, the pericardium opened, a snip made in the ventricle, and the blood received on a clean slide or cover-glass. A readier way, and one which answers nearly as well, is to decapitate the frog and receive the blood as it flows from the vessels of the neck. Or the blood may be allowed to flow into a watch-glass and to coagulate. If the coagulum be taken in a pair of forceps and the slide touched with it, a drop of serum containing numerous corpuscles (fewer of course than in the fresh blood) will be got. In any case the drop should be large enough to fill the whole space between the cover-glass and the slide, but only in such a thin layer that

the corpuscles do not lie one over the other. Examine with the high-power. Notice the pale-yellow oval bodies covering the field (red corpuscles); in most of these no nucleus is apparent, but in some a faint granular oval spot in the centre is already visible, and will gradually become more distinct. If the drop be large, some red corpuscles will probably lie so as to present their edges to view, and will appear linear, with slight bulging in the region of the nucleus. The corpuscle is, therefore, not oval like an egg, but a flat oval. Observe the white corpuscles, much fewer than the red; colourless, of somewhat variable size, but all much smaller than the red. At first all are spherical, and, however they lie, will present a circular outline. Subsequently many will assume an irregular shape, which varies from moment to moment (amœboid movements). In some corpuscles the granules are coarse and evident; in others so fine as not to be separately distinguished by ordinary powers. In most white corpuscles no nucleus is visible in the recent preparation.

2. As the preparation has probably coagulated, and in consequence reagents cannot well be applied, it is best to make a fresh preparation as in 1. Place on the slide, touching one edge of the cover-glass, a small drop of water, which will flow in by capillarity, and at first drive the blood before it, but the fluids mix gradually from their line of junction. Notice how, in the current produced by the influx of the water, the red corpuscles roll over and over, presenting alternately an oval and a linear shape. Observe how, as the water gradually mixes with the blood, the corpuscles become spherical, appearing round and of less diameter than the long axis of the original oval; how the nucleus becomes visible; the diffusion of the colouring matter and the gradual fading and disappearance of the corpuscle leaving the nucleus. Observe that after the action of the water the blood has become transparent or laky.

3. Mix on the slide, with the point of a needle, a drop of recent frog's blood, and an equal-sized drop of 10 per cent solution of common salt. Observe how the blood becomes of brighter colour and more opaque than before. Cover, remove any excess of fluid from the edges of the cover-glass with filter-paper, and examine. Observe that the corpuscles have lost their smooth homogeneous structure and are somewhat granular; that their outline is harder and more defined than before; that many of them have a crumpled or folded appearance, frequently showing itself as a plication radiating from the nucleus; that the nucleus is visible; that the colouring matter has not diffused.

4. To the same preparation add a drop of magenta staining fluid.* There is at first a good deal of precipitation, but on

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* Crystals of magenta or fuchsin dissolved in alcohol and largely diluted with water.

continuing to run in the dye the corpuscles become stained. Remove with filter-paper any magenta remaining at the side of the preparation; run in from the same side a stream of water. This washes away the dye and leaves the stained corpuscles on a clear ground. The nucleus is intensely stained. A well-defined outline or cell-wall* becomes visible, on the inner side of which a number of little stained masses are seen. The corpuscle between the cell-wall and the nucleus is unstained.

5. **Human Blood.**—From a puncture in the finger squeeze a drop of blood. Place it in the middle of a cover-glass and quickly apply this to the slide. It is of great importance to have the layer of blood very thin. Notice that the red corpuscles, here much smaller and more numerous than in the frog's blood, present either a round or a linear shape according as they lie flat or on edge. When in the latter position it can be seen that the middle part of the corpuscle is thinner than the margins, and that these are rounded so that the figure is somewhat dumb-bell-shaped. Observe as the corpuscles move about among one another that they readily alter their shape when they meet an obstacle, but immediately resume their natural appearance when this is past, thus manifesting their flexibilty and elasticity. Notice in the centre of those corpuscles which lie flat a badly-defined spot, which becomes bright or dark as the fine adjustment is altered. This must not be confounded with a nucleus. It is an effect produced on the light by the concave centre and rounded edges of the corpuscle. Shortly after the preparation is made many corpuscles will arrange themselves in rouleaux. Towards the edge of the preparation spinous corpuscles may be seen. Microcytes may be present. Notice the white corpuscles, very few, two or three in each field; slightly larger than the red, without colour, and of granular appearance, standing by themselves after the rouleaux are formed, and stationary while the red corpuscles are moving. The white corpuscles remain at rest partly because they are larger than the red and support the cover-glass, partly because they are of a tenacious consistence, and cling to whatever surface they come into contact with.

6. Make a fresh preparation of human blood, and allow water to flow in under the cover-glass as in 2. Observe the corpuscles as they roll in the current, the white corpuscles remaining fixed. As the water acts, notice how the red corpuscles become spherical: try to see the bowl-shaped stage in which the corpuscles are concavo-convex. Observe the diffusion of the colouring matter, and how the corpuscles become gradually fainter and at last disappear: observe particularly that at no stage in the process is any nucleus visible. The white corpuscles persist after the water has acted.

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* This apparent cell-wall is produced by the action of the reagent on the surface of the corpuscle.

7. Mix on the slide equal drops of fresh human blood and 10 per cent. salt solution. Observe the increased brightness of colour and greater opacity: cover and examine. There are now no rouleaux. The corpuscles are more opaque than before and slightly granular, with hard outline and distorted crumpled shape. No diffusion of colouring matter.

8. Irrigate the same preparation with $\frac{1}{2}$ per cent. tannic acid solution, made by dissolving the tannic acid in warm water and filtering the solution. When the acid comes in contact with the corpuscles, there starts out from near the edge of each one or more little knobs or longer tail-like projections, appearing bright and containing most of the colouring matter of the corpuscle. The outline of the corpuscle becomes well defined, and is formed by a distinct line (artificial cell-wall). Try to see the sudden formation of the projections.

9. Same preparation. Remove with filter-paper any tannic acid which has not already run in, and from the same side run in magenta: follow this, when the preparation is stained, by a stream of water, to remove the excess of dye. The projections are stained a bright red. The remainder of the corpuscle is unstained.

10. The effects of heat on the blood may be readily shown by the ingenious method of Ranvier. Make a preparation of fresh human blood. Heat in the flame of a spirit or gas lamp a thin stick of solder until it just begins to melt. Then touch the centre of the cover-glass with the melting metal. If a drop comes off the rod it must be allowed to cool before it is detached from the glass. It will now be found that under the spot where the solder was applied the corpuscles are completely destroyed and the hæmoglobin set free. Surrounding this spot there is a zone where the corpuscles are changed into little spherical bodies of various sizes, mixed with which will be found corpuscles with long tail-like appendages, and presenting other alterations of shape; while outside this the corpuscles will appear of their normal shape, and lie in rouleaux. In order to see the changes caused by heat actually taking place, and to determine the temperature at which they occur, it is necessary to examine the blood on a heated stage, of which there are various forms in use.

11. Dilute on the slide a drop of recent blood of a guinea-pig or rat with enough water to make it laky. Leave it uncovered until it begins to dry about the edges: then cover and examine from time to time. Crystals of hæmoglobin will form as the fluid evaporates. These will have the shapes peculiar to the animal from which the blood has been taken. Defibrinated blood may be kept sealed in glass tubes for an indefinite time without undergoing more than a very limited putrefaction. The red colour of arterial blood soon changes to dark purple, and the spectroscope shows that all the hæmoglobin is reduced. Such

blood, when removed from the tube and diluted with water, crystallizes with great readiness and in large crystals. It is convenient to keep blood sealed up in this way in several small tubes, one of which can be opened when crystals are required (Gscheidlen).

12. Take a small fragment of dried blood, recent or old; rub it to powder on a slide, and mix it with a minute quantity of powdered salt: put on a cover-glass and run in glacial acetic acid until the space between the glasses is full; then heat cautiously over a spirit or glass flame until the acid begins to boil. Allow the preparation to cool, and if the acid has evaporated, so as to leave an empty space under the cover-glass, this may be filled by running in water. Numerous dark-brown rhomboidal crystals of hæmin will be seen, of various sizes, and lying singly or in groups.

13. Make a preparation of recent frog's blood. As the observation must last some time, and as it is important that the liq. sanguinis should remain unaltered, it is well to seal up the preparation by painting around the edge of the cover-glass a layer of oil, which must extend about $\frac{1}{8}$ inch over the surface of the cover-glass, and at least the same distance on the slide beyond this. Or the preparation may be sealed up with paraffin applied with a heated wire. Look out for a large white corpuscle which lies by itself. Choose one which is finely granular, and which has an irregular shape. Watch it closely, and it will be observed to undergo changes of form, at first slow and of slight extent, but increasing as the corpuscle recovers from the violence done to it in making the preparation. In order to make sure of the movement, the shape of the corpuscle should be sketched every half minute or so. In this way a series of very different figures will be got, which can be subsequently compared with one another. If finely divided particles, as vermillion or aniline blue freshly precipitated from its alcoholic solution by water, be suspended in $\frac{3}{4}$ per cent. salt solution and mixed with the blood, the white corpuscles may sometimes be seen to take into their interior some of the granules. This is called feeding the corpuscles. If salt solution, in which vermillion has been rubbed up, be injected into the lymphatic sac of a frog, in a few hours the white corpuscles of the blood will contain numerous coloured grains. If, instead of vermillion, milk be injected, the corpuscles will contain fat globules and resemble closely the so-called colostrum corpuscles of milk. If a piece of elder pith or other porous substance be introduced for a few hours into the lymphatic sac of a frog, and after its removal fine sections be made, the lymph corpuscles will be found to have penetrated to a considerable distance into it. This they do by virtue of their power of performing spontaneous movements. These experiments, however, although in the highest degree instructive, cannot be performed by students. In order to study the amœboid movements of the

corpuscles in the blood of a warm-blooded animal, the preparation must be examined at the temperature of the body on the heated stage.

14. Place a *large* drop of blood on the slide, and cover. Put the preparation on a support under a bell glass standing in a plate into which some water has been poured. In this space, which is completely saturated with watery vapour, no evaporation from the preparation can occur. This arrangement is called a **moist chamber**. It will be frequently referred to. In about an hour or less remove the slide and place it carefully in a saucer of water; remove the cover-glass under water, taking care not to detach from the slide the film of coagulated blood. Allow this to lie in the water for some minutes until most of the colouring matter is dissolved out. Then remove the slide with the adherent film, now almost colourless. Wipe the slide dry, and remove all excess of water from about the film with filter-paper, taking care not to touch the object with the paper lest they should stick to one another. Then apply a cover-glass. Observe the beautifully fine reticulum of fibrin; the little granular masses at the nodal points; the few white corpuscles and decolorised remnants of red corpuscles in the meshes of the network.

15. To see the third corpuscular element, a cover-glass is fastened down tightly to the slide by two strips of adhesive plaster. Some blood from a puncture in the finger is allowed to run in between the glasses. In places the corpuscles will be crowded together and the liq. sanguinis drained off. Here the colourless bodies may be seen, either merely as round spaces, about which the red corpuscles are moulded, or their faint outlines may be more or less clearly visible (Norris).

Or, a drop of 0·75 per cent. salt solution coloured with methyl violet is placed on the finger, and a puncture made through the drop so as to cause the blood immediately at its exit to come into contact with the coloured salt solution. The two fluids are mixed with a needle on the finger, and a drop of the mixture examined. In such a drop, pale, colourless discs, smaller than the red corpuscles, may be seen (Bizzozero).

16. Permanent preparations of blood are made with difficulty. A very thin layer of blood may be dried rapidly on the slide by heat, or by blowing on it a current of air. The corpuscles adhere to the glass and retain their shape fairly well. Such a preparation is covered dry and sealed in the usual way.

A better method is to place the blood on the slide, and invert this for a few minutes over the mouth of a bottle containing a solution of osmic acid 1 per cent., the fumes of which fix the corpuscles, and prevent their undergoing further change. A small drop of a saturated solution of acetate of potash is placed on the cover-glass, and this is inverted and placed on the drop of blood.

The excess of fluid is removed with filter-paper, the slide dried, and the preparation sealed (Lankester).

Hæmoglobin crystals are best preserved by rapid drying on the slide; a small drop of oil of cloves is added, and the preparation mounted in Canada balsam or Dammar varnish.

Hæmin crystals can be preserved either in glycerine or balsam.

Hæmatoidin crystals can be put up in glycerine.

It is best for students not to begin to make permanent preparations until they have gained some proficiency in the manipulation necessary in preparing temporary objects.

CHAPTER IV.

EPITHELIUM AND ENDOTHELIUM.

ALL the free surfaces of the body are covered by a tissue which consists of cells only, or, more accurately, of cells held together by an extremely small quantity of a cementing material, as the stones of a wall are by the mortar. Into this tissue blood-vessels never enter, although in many cases nerves ramify freely among the cells.

The free surfaces which we meet with are of two kinds: one forms part of the true covering of the body, or did so at some period of life. As examples, may be given the skin, mucous membranes of digestive, respiratory, genito-urinary tracts, the lining of the ventricles of the brain, and the central canal of the spinal cord. The cells which cover these surfaces are called **epithelium**.

The second class of surfaces never at any period communicated with the exterior or formed part of the true covering of the body, but bound closed cavities in its interior. Examples of this class are to be found in the serous membranes, the interior of the heart, blood-vessels, and lymphatics, sheaths of tendons, and articular cavities. The cells covering these surfaces are called **endothelium**. While the epithelia are developed for the most part from the upper or lower germinal layers of the embryo, the endothelia are formed from the middle layer, and their relationships are consequently rather with the connective tissues than with the epithelia.

The terms epithelium and endothelium are not always used in the sense just defined. Some writers reject the term endothelium altogether, and call all the cell tissues epithelium: others employ the term endothelium to indicate those tissues which consist of a single layer of flat cells, whether these form part of the surface of the body or not. In this sense certain structures which are generally described as epithelial are classed among the endothelia, as, for instance, the cells which cover the alveoli and infundibula in the lungs.

1. **Epithelium.**—Since the surface of the body is covered by epithelium, this structure must form a continuous layer, except in a few places where in process of development portions of the general surface, together with their epithelium, have become separated from the remainder, and now bound closed cavities. Examples of this occur in the cavities of the brain and spinal cord, the internal ear, and the space between the two layers of the retina, where, although the space is obliterated, the epithelium persists. Elsewhere the epithelium forms a continuous sheet, although much involuted, for not only does it follow the general surface of the skin and mucous membranes, but it passes down into all the glands which open on the surface, and lines their ducts and secreting cavities.

Although having many features in common, the cells forming this epithelial sheet differ considerably in different parts: these differences depend on the facts—(1) that the cells sometimes lie in a single layer, sometimes in several superposed layers, and (2) that the shape and arrangement of the cells is very different in different parts. The different kinds of epithelium pass into one another by transitions which are sometimes abrupt, sometimes more gradual.

In consquence of the differences just mentioned, the epithelia have been classified—A, according to the number of layers of cells present. 1°. into those which consist of one layer of cells or **simple epi-**

thelia; 2°. into those which consist of many layers, or **stratified or compound epithelia.**

B—According to the shape of the cells. 1°. when these are flat and lie with their surfaces parallel to the surface they cover, the epithelium is **scaly** or **squamous**; 2°. when the cells are long, prismatic, or conical bodies, which are arranged so that their long axes are placed at right angles to the surface they cover, the epithelium is **columnar** or **cylindrical**; 3°. a third species of epithelium occurs which—because its cells are of very irregular shape, some resembling those of scaly, others those of columnar, and because it is frequently found at points where scaly and columnar epithelium meet—is called **transitional epithelium.**

Scaly epithelium may be simple or compound; transitional epithelium is always compound; while columnar epithelium is (in a sense to be afterwards explained) nearly always simple.

Epithelial cells are sometimes provided with fine hair-like appendages on their free surface. These appendages or cilia are continually in motion, and cause a current in the fluid which covers the surface of the mucous membrane. Epithelia which have such appendages are called **ciliated**: transitional epithelium is never ciliated; scaly rarely in the human body, while large tracts are covered by columnar ciliated epithelium.

The epithelia which occur in the interior of glands, and constitute their secreting elements, are too variable in structure and arrangement to admit of classification, and will be described separately with each gland. It may be said here, however, that the differences in shape of the **glandular epithelial** cells is of less importance than the differences in their structure and chemical constitution.

In the organs of special sense the epithelial cells undergo peculiar modifications of shape and structure, and form the organs in which the nerves terminate. This is **neuro** or **nerve epithelium.**

In many cases the epithelium rests on a thin membrane, separable from the subjacent parts. Such a 'basement membrane' appears homogeneous, but consists really of flat cells joined closely, edge to edge, and there is some doubt as to whether it belongs to the epithelium or to the deeper parts. In other cases no such basement membrane is to be seen, but the epithelial cells rest directly on the tissue of the mucous membrane, which may be somewhat condensed near the surface.

Prolongations of connective tissue cells among those of the epithelium have been described in several mucous membranes (Klein); and amœboid, wandering cells are frequently found in the cementing substance.

The following Table gives a summary of the different epithelia, and the parts of the body in which each is found :—

EPITHELIUM.

- 1. Columnar.
  - **Ciliated.** Nose; lachrymal passages; antrum and frontal sinuses; upper part of pharynx; larynx and bronchi; Eustachian tubes; Fallopian tubes and uterus; epididymis, and lower part of vas deferens; central canal of spinal cord; ventricles of brain.
  - **Non-ciliated.** Digestive tract from cardia to anus; ducts of most glands; upper part of vas deferens; vesiculæ seminales and middle part of male urethra; surface of ovary.
- 2. Transitional. Pelvis of kidney: ureter; bladder; upper part of male and of female urethra.
- 3. Scaly.
  - Simple.
    - **Ciliated.** Ventricles of the brain; tympanum.
    - **Non-ciliated.** Choroid plexus; interior of semicircular canals; part of tympanum; some finer ducts; alveoli of lungs; post: surface of anterior capsule of lens.
  - Compound. Skin: cornea and conjunctiva; mouth; lower part of pharynx; œsophagus; epiglottis and edges of true vocal cords; vagina; lower part of male and female urethra.
- 4. Glandular.
- 5. Nerve.

I. **Scaly Epithelium.**—1. **Simple Scaly Epithelium** consists of a layer of thin, flat cells, lying side by side—not overlapping, but meeting edge to edge. It resembles endothelium very closely, and occurs in so few places that its description, and the methods for its study, will be given when these parts are being described.

2. **Compound Scaly Epithelium** consists, from its deepest to its most superficial part, of the following layers: 1. A layer of columnar cells, resting by one end on the basement membrane, with which they often indigitate by fine teeth. In some cases these cells are short, almost cubical, with badly-defined outlines, and large nuclei in proportion to the amount of protoplasm: in other cases, as on the anterior surface of the cornea, they are tall, distinct, club-shaped cells, occupying a very considerable proportion of the whole depth of the epithelium. 2. Several layers of irregular-shaped cells: these present ridges and depressions, where they fit one against the other, and against the upper ends of the cells of the first layer; they consist of a granular protoplasm, and each contains one or two nuclei. Their surface is covered with very minute spines. It was at first supposed that the cells dovetailed with one another by these spines, like cogged wheels, but it is now known that the spines meet point to point, and that the cells are continuous one with another by little bridges, very much as postage stamps are, across the line of perforation. When these bridges are torn across in isolating the cells, the fragments which remain adherent to the cells form the spines. The cells become more and more flattened as they approach the free surface, and pass gradually into the third layer, which consists of thin, flat scales, in which the greater part of the protoplasm has undergone transformation into a transparent, structureless, horny material. Each cell contains a shrivelled nucleus, around which are a few granules. These cells lie in several layers, the cells not meeting by their edges,

but overlapping one another. They have no spines, and are so loosely attached to one another that the superficial cells are continually undergoing desquamation. The degree to which the horny transformation of the cells is carried differs in different epithelia.

While the general character of the compound scaly epithelium is the same wherever it is met, the thickness of the second and third layers is subject to great variety.

II. **Transitional Epithelium.**—This variety of stratified epithelium consists of three layers. Resting on the basement membrane by their smaller ends are club-shaped cells, whose large rounded extremities are directed towards the surface. Between the deeper ends of these cells, and filling up the interstices which here exist, are others of smaller size and very irregular shape. Covering over all, and forming the most superficial layer, are large, flattened, but thick, cells, on whose under surface are a number of deep rounded depressions, into which fit the heads of the club-shaped cells. The depressions are separated by prominent ridges, which appear as bright and curved lines, when the isolated cells are seen from their under surface. All the cells of this epithelium possess a granular protoplasm. In the two deeper layers each cell has one nucleus. In the club-shaped cells this is oval, the long axis being in the long axis of the cell. The superficial cells often contain several nuclei. The above description is taken from the epithelium of the bladder, when this viscus is contracted : when it is distended all the cells are flat, and the whole epithelium is thinned, each cell having to cover a much larger area than before.

In the transitional epithelium of other parts the superficial cells are much smaller than in that of the bladder.

III. 1. **Columnar Non-ciliated Epithelium.**—The cells of this epithelium are for the most part long conical-shaped bodies placed vertically to the surface, resting by one of their ends on the basement membrane. Each cell consists of a granular protoplasm, and contains an oval nucleus, situated about its middle: in some cases the deeper part of the cell is very distinctly striated in a longitudinal direction. The lower or attached end of the cell sometimes expands into a conical-shaped foot; sometimes divides into several fine processes; and sometimes passes into a sort of beak, which is bent at right angles to the general direction of the cell, so that these beak-like processes of neighbouring cells come to overlap one another.

The sides of the cells are frequently marked by depressions and ridges, by which they correspond to the projections and hollows of neigbouring cells. The free surface has in many places a shining border, which can sometimes be detached as a continuous cuticular membrane over the extent of several cells. With good lenses, and in favourable specimens, this border is seen to have very fine radial striæ, which have been supposed by some observers to be pores, by others to indicate that the border is constituted of minute rods, but whose true import cannot yet be considered as settled.

Lying between the completely formed conical cells are others, which fill up the intervals between their smaller ends, and which must be looked on as young cells destined to replace the conical cells when they are cast off. These young cells are sometimes short pyramidal bodies, whose broader ends rest on the basement membrane between the attached ends of the adult cells; sometimes long spindle-shaped bodies, whose attached ends may have one or other of the forms described above, and whose small free end terminates generally just below the surface between the conical cells. The young cells are called **replacement cells.**

Columnar epithelial cells frequently undergo a remarkable change. The portion of their protoplasm next the surface becomes converted into a colloid substance which distends the cell, probably in consequence of its attraction for water. Owing to this swelling the cell changes its original shape, and becomes something like that of a wine-glass whose foot is removed; hence these cells are called **goblet cells.** A further consequence of the swelling is, that the nucleus and the remains of the protoplasm are pushed down towards the base of the cell. The free end finally gives way, and the colloid mass is discharged as mucus, leaving the upper part of the cell empty, or traversed only by a scanty network of protoplasmic threads. The opening through which the discharge of mucus takes place is generally narrower than the dilated deeper part of the cell, and is frequently provided with an everted edge. The shape of the goblet cells varies a good deal according to the degree to which the protoplasm undergoes the transformation into mucus, prior to the discharge of the latter. If only the upper part of the protoplasm changes, the cell is of a true goblet shape; but if the process extend further, the entire cell may become spherical or oval, and the remaining protoplasm and nucleus form a narrow crescentic figure at its deeper part. Goblet cells occur without order among the other cells, and their number varies greatly at different times. They must be looked on as unicellular glands; and in many parts, as in the stomach and intestines, they are the sole source of the mucus which covers the surface of the mucous membrane.

The process of formation of goblet cells proves that columnar epithelial cells possess a cell-wall firmer than, and of different chemical composition to, the contained protoplasm.

2. **Columnar Ciliated Epithelium.**—This epithelium in the general shape of the cells resembles very closely that last described. The cells are conical with their attached ends pointed, branched, or

terminating in an expanded foot. Their nuclei are oval, and between the mature cells are younger forms described in the last section. This form of epithelium differs from the last, in that the free end of each of the mature cells bears a number of minute hair-like processes called **cilia.** In the lower animals there are cells with only one cilium ; but in the higher classes each cell has from 10 to 30 cilia. Immediately under the cilia the cell possesses a thick shining border, which, with moderate powers, presents a beaded appearance. It was supposed that this was a cuticulum perforated by holes through which the cilia passed, to be rooted in the protoplasm of the cell. It has, however, recently been shown that the apparent cuticulum is really composed of a number of short rods, to each of which a cilium is attached, not directly, but by a narrow intermediate portion. The basal end of the cilium next to this is bulbous. The bulbs of all the cilia lying together appear as a dark line ; on this follows a clear line corresponding to the narrow intermediate portions, and then the bases of the cilia form the shining border seen at the free end of the cell. From these bases fine threads can sometimes be seen continued into the protoplasm of the cell. These processes have been seen to coalesce into a single thread, which, passing by the nucleus, is continued into the deeper part of the cell. It is not very clear what the nature of these threads is ; they are neither contractile nor nervous (Engelmann).

During life the cilia are always in motion, and by this means a current is maintained over the surface of the membrane. The direction of this current is, in man, always towards the outlets of the body. The motion of the cilia consists in a rapid bending in one direction, followed by a slower movement in the opposite. These movements take place at each side of a line inclined to the axis of the cell in the direction of the more rapid movement, in which direction the current is produced in the fluid above the cilia. The cilia in some cases bend at their bases, in others about their middle. When they come to rest they assume a position inclined to the surface in the direction of the more rapid bending (Ranvier).

All the cilia do not move simultaneously, but the motion can be seen to move along from one cell to another in a wave. It is impossible to follow the movements of the individual cilia when the motion is active, but as the cell is dying, or exposed to influences which affect the movements, these become slow, and can be readily studied. Acids stop the movements of the cilia, while weak alkalis favour them. If a current of carbonic acid, or of air impregnated with chloroform vapour, be passed over the cells, the cilia gradually cease to move, but begin again when fresh air is supplied. This experiment may be repeated many times with the same preparation.

If the cells be examined on the warm stage, and gradually heated, the movements of the cilia are at first hastened, and then come to rest in strong flexion. If the temperature be not too high or long continued, the cilia will recommence their movements when it is lowered (heat tetanus); but if the high temperature be maintained or increased the arrest is permanent (heat rigor). The temperature at which these phenomena occur differs for different classes of animals, and in general corresponds to that at which tetanus and rigor occur in the muscles. A very low temperature suspends the movements of the cilia.

Between the ciliated cells, goblet cells are found in variable number, and do not differ from those already described.

Endothelium.—Endothelial cells, although differing greatly in form and size in different places, resemble each other much more than do the different kinds of epithelium. They are always flat cells of great thinness and delicacy. Each of them contains a flat nucleus, about which the cell is somewhat thickened, so as to have, when seen in profile, a spindle shape. The cells lie always in a single layer, meeting by their edges, and not overlapping. Their shape may be polygonal, as in the serous membranes ; very long and narrow, as in the blood-vessels ; or quite irregular, as in the smaller lymphatic. The edges of neighbouring cells always accurately correspond, however uneven these edges may be. Between the cells there is a small quantity of an albuminous cementing substance, which reduces nitrate of silver, and in preparations treated with this reagent appears as fine black lines, marking out the boundaries of the individual cells.

In silver preparations there appear frequently between the ordinary cells others, which are peculiar in being smaller and in having no nucleus. These are called **intercalated cells**.

In the serous membranes there are found openings between the endothelial plates. These are commonly surrounded by small cells, which are granular, and stain brown in silver. About these openings the ordinary endothelial cells are frequently arranged in a radiating manner. The openings are called **stomata**. By means of these the lymphatic vessels communicate with the serous cavities. They are very numerous in some places, as on the peritonæal aspect of the diaphragm, and on the membrane which forms the posterior wall of the abdomen in the frog (septum cysternæ lymphaticæ magnæ).

In some membranes, more particularly those which are not continuous, but retiform or fenestrated, as the omentum of the dog or guinea-pig, or the mediastinal pleura of the cat, there are found in places groups of cells, which are small, granular, lie in heaps, and stain deeply with silver. These patches are often of considerable size, so as to be readily visible to the naked eye, as white points or streaks.

The individual cells readily detach themselves, and are found free in the fluid of the cavity. This modification of endothelial cells occurs much more extensively when there has been chronic inflammation of the serous membrane. It is called **germinating endothelium** (Klein). In some few instances endothelial cells have cilia. This is the case in the abdominal cavity of the female frog, more particularly at the breeding season. Among the ordinary endothelial cells are found tracts and patches of smaller cells which are ciliated. The currents produced by the movements of the cilia are supposed to favour the passage of the ova towards the open mouth of the oviduct. In the peritonæum of the male frog also ciliated endothelium exists, but less abundantly than in the female.

It is necessary in the practical study of epithelia to proceed in two ways:—1°. To separate the cells one from the other, so that they may be seen from all sides, and their shape and structure submitted to examination; 2°. To examine the cells *in situ*, as they lie together, so as to make out their relations to each other and to the neighbouring parts. The first is accomplished by methods of isolation; the second by sections. It is the first only which will be attempted here: the sections will be made afterwards, when the organs to which the different epithelia belong are under examination.

If the surface of a fresh mucous membrane be gently scraped wtih a scalpel, there comes off a pulpy material which, when diffused in a drop of salt solution and examined, will be found to consist mainly of epithelial cells. When these are of firm consistence and loosely attached, such a method is sufficient for their preparation. This is the case with the superficial cells of the compound squamous epithelia.

1. Scrape lightly with a penknife the inner side of the cheek. With a needle distribute a small quantity of the scraping in a drop of 0.5 per cent. salt solution on a slide, so that there may be no large adherent masses visible to the eye. Cover and examine with the high power. Observe the **flat scale-like cells**, lying singly or in groups, in the latter case overlapping one another by their edges. Each cell is of transparent homogeneous appearance, and has one small oval nucleus near its middle, sometimes two. About the nucleus are a number of coarse granules. The cell is frequently folded or wrinkled, and at the folds its extreme thinness can be seen. If the prepara-

tion be irrigated with magenta, and the excess of staining fluid then removed by irrigation with water, the cells are stained red, and the nucleus a much deeper red.

These cells may be preserved permanently by diffusing the scraping of the cheek in a drop of 1 per cent. picrocarmine, covering, and allowing the preparation to lie in the moist chamber until the cells are stained—the nuclei pink, the cell itself yellow. Then a small drop of glycerine is placed at the edge of the cover-glass, and allowed to diffuse in gradually and mix with the picrocarmine. The excess of fluid is removed with filter-paper, the slide wiped clean, and the preparation sealed up in the usual way.

In most cases, however, mere scraping of the fresh mucous membrane gives very imperfect results, because the cells are so soft and fragile, and the cementing substance holding them together is so firm, that in the scrapings only imperfectly separated and broken cells are got. It is advantageous, therefore, to submit the mucous membrane to some reagent which will increase the consistence of the cells, while it softens or dissolves the cementing substance. Such reagents are called **macerating fluids.**

The following is a list of the more important and commonly used macerating fluids, and, approximately, the time they require to act:—

1. Müller's fluid (bichromate of potash 2½, sulphate of soda 1, water 100), diluted with an equal part of water; 24 hours to a week.
2. Neutral chromate of ammonia, 5 per cent. solution; two or three days.
3. Chromic acid, 1 part in 10,000 water; two or three days.
4. Dilute alcohol (strong spirit 1, water 2); 24 hours.
5. Osmic acid (1 part in 1000); one or two days.
6. Iodine serum (the amniotic fluid of cow or sheep, got fresh, filtered, and iodine added to give a yellow colour. It must be kept in a cool place, and fresh iodine added as the colour fades); 24 hours to two days.
7. Caustic potash, 40 per cent. solution; ten minutes to half an hour.

Of these 1 and 4 are most generally useful for epithelia. 6 is most valuable for many purposes, but is difficult to get and to keep good. 7 requires special precautions, which will be explained in another place. 2 is very useful for isolating the elements of many glands: 3 and 5 for separating the elements of nerve centres.

2. Place the tongue of a rabbit or guinea-pig in some ounces of diluted Müller's fluid for about a week. The superficial horny scales come off readily *en masse* as a membrane. If the under surface of this membrane, or the surface of the tongue,

exposed after its removal, be scraped, a soft material is got, which can readily be diffused in a drop of water, and consists of the **deeper cells of the compound squamous epithelium.** Observe the cells, polygonal and very irregular in shape, with ridges and depressions, and in favourable specimens with very fine spines projecting from their surface. In young animals these cells have frequently two nuclei. Permanent preparations may be made by first washing the macerated membrane for a considerable time in water, frequently changed until it ceases to take up colour, then isolating the cells in picro-carmine, and proceeding as directed in 1.

3. Lay open the bladder of a recently killed mammalian animal, and place it in diluted Müller's fluid, or diluted alcohol, for two or three days. Scrape the surface: examine the scrapings in water. Observe the great variety in size and shape of the cells of the **transitional epithelium.** The large superficial cells are the most striking objects. They present themselves either in profile, when the deep concavities on their under surface, separated by prominent ridges, will be seen; or in face, when the ridges will appear as curved lines, becoming bright on raising the tube of the microscope by the adjusting screw. There will also be found the long club-shaped cells, with their rounded ends, and their sides frequently indented by impressions of the third or small cells, which fit in between their deeper ends. Patches of epithelium will not unfrequently be found where all the layers of cells lie together retaining their natural relations.

Permanent preparations as in 2. If alcohol has been used for maceration the staining can be effected at once. If Müller's fluid, the membrane must first be washed until the water is no longer coloured.

4. Macerate in Müller's fluid or dilute alcohol for two or three days a portion of the small intestine of a frog or mammal. Scrape the surface, and diffuse the scrapings in water. The **conical-shaped cells** are seen, each with an oval nucleus, and a shining thickened border, in which a fine striation may possibly be detected with the high power. The lower end of the cell may be pointed, branched, or continued into a beak. If the animal is killed during digestion the cells may contain fat drops. **Goblet cells** may be found. Patches of cells not separated from one another often occur, and present their outer small ends to view. Observe the mosaic formed by these, interrupted now and then by round openings, the mouths of the goblet cells. On focussing for the surface no nuclei are visible, but on depressing the tube a nucleus is seen in each cell, and the openings corresponding to the goblets increase in size. This is due to the fact that the goblets are narrow at their openings, but swell out on a deeper plane.

If the stomach be treated as above, almost all the cells will be found to be goblets.

Permanent preparations of columnar epithelium macerated in alcohol, or Müller's fluid, may be prepared as described under 1 and 2.

5. Decapitate a frog just behind membrana tympani. Cut away the lower jaw and tongue. Slit open the nares, and place the head in dilute Müller's fluid. Dissect out the œsophagus, slit it open, and place it also in the Müller's fluid. The trachea of a recently killed rabbit or cat may be similarly treated. After a couple of days examine scrapings. In those from the mouth and œsophagus of the frog observe the **conical ciliated cells**, broad above, with numerous long cilia; the shining border under these, the oval nucleus, and generally pointed lower end. The goblet cells are very large and numerous. In very many cases the whole cell has assumed a spherical or oval shape, with a narrow opening above, the protoplasm, with the nucleus, forming a thin crescent at the lower part.

In cells got from the nares of the frog the cilia are very long and distinct, and in good preparations the shining border can be seen to be made up of rods, each of which is attached by a narrow portion to a cilium, which is swollen into a bulb at the point of attachment. In very favourable specimens continuations from the cilia into the protoplasm of the cell can be seen. Mixed with ciliated and goblet cells are other cells of great length and different shapes. These belong to the olfactory epithelium, and will be described further on.

In the mammalian trachea the cells are more slender, and carry fewer cilia than in the frog. The lower ends of the cells are frequently divided. The goblet cells are fewer and smaller than in the frog; and spindle-shaped, and small conical replacement cells are frequent.

Permanent preparations as before.

6. To see **ciliary motion**, scrape the palate of a recently killed frog, and with needles separate the product in 0.5 per cent. salt solution. Place a hair under the cover-glass, so as to keep pressure from the cells. These will for the most part not be completely separated, but will lie in groups. Some will have their ciliated surface turned upwards; others will lie on their sides. The movement of the cilia will be at first rapid, but after a time will become slower, and finally cease, continuing longer near the margin of the preparation than near the centre, where the cells are further removed from the oxygen of the air.

If the thin free end of the tongue of the frog be cut off, and carefully spread out on the slide, and covered in a drop of salt solution, the ciliary motion of the cells along its border can be beautifully seen, the direction of the current being indicated by any free particles in the fluid, which are rapidly carried along by the side of the preparation. Observe the wave-like character of the motion.

The best object, however, on which to study ciliary motion is the gill of the oyster or mussel. The gill consists of lamellæ placed one over the other, like the leaves of a book. Each lamella is marked by deep grooves running radially towards its free edge. The sides and surface of the projections bounding these grooves are covered by cells, whose cilia are so long that they can readily be seen with quite a low power.

Cut off a portion of one of the lamellæ, and gently separate the linear projections with needles, touching them in as few places as possible. Put on the preparation a drop of the fluid which is contained in the shell of the animal, and examine with a low power (half to quarter inch), without applying a cover-glass. On the separated threads the motion of the cilia can be distinctly seen. Notice that the motion along the two sides of the same groove is in opposite directions—down one side, up the other.

Put a few drops of chloroform in the bottom of a bottle whose cork is perforated by two glass tubes, to one of which a piece of India-rubber tubing is attached, and the other of which is bent, so that its end can be placed near the object under the microscope. Air blown through the bottle will emerge charged with chloroform vapour. Blow gently through the India-rubber tube, having brought the opening of the other tube close to the uncovered object. In a few seconds the ciliary movement will become slow and cease, not everywhere at once, but irregularly over the preparation. When the motion has ceased, remove the chloroform bottle, and blow air on the slide. The movements will recommence at first here and there, and will soon become general. While the movement is slow its details can readily be followed.

A similar stoppage can be produced by passing over the preparation a current of carbonic acid, the movement recommencing when air is again supplied.

Having studied these phenomena on an uncovered preparation, apply a cover-glass, and examine with a high power.

Pieces of apparatus are made by which gases can be applied to microscopic objects while they are under examination by the highest powers, and are, of course, covered by a cover-glass. Such instruments are called *gas chambers*, and are indispensable for many purposes. The above arrangement is, however, quite sufficient for the needs of a beginner.

The effects of heat on ciliary motion can be studied only on a warm stage.

7. Endothelium is usually studied by what is called the **silver method**. This gives admirable results, but requires a good deal of care in its application. The following description

applies only to the serous membranes. The methods for the blood-vessels, lymphatics, &c., will be subsequently given.

Suppose the omentum is to be prepared. The animal is killed by hæmorrhage, the abdomen opened, the omentum carefully raised by forceps, cut off from the stomach with sharp scissors, and transferred at once to a large capsule containing a solution of nitrate of silver of the strength of one-half per cent. The membrane is moved about gently in this fluid, so as to be everywhere completely wetted by it. It soon assumes an opaque milky appearance, which shows that the silver has acted sufficiently. It is then removed to a vessel containing distilled water, in which it is moved about, so as to remove any silver solution adhering to it. It is then transferred to fresh water, if it is to be immediately mounted, or to spirit if it is to be kept, and exposed to the light in one or other of these fluids. It soon becomes brown, and is then fit for mounting, and must not be further exposed to light. If such a silvered membrane be kept in water the cells fall off, but it keeps indefinitely in spirit.

Before describing the method of mounting we shall give the plan to be followed in preparing the mesentery of a cat or rabbit, and the serous membranes of the frog. The abdomen of the cat or rabbit, which has been killed by hæmorrhage, is opened, and the intestines are gently separated, so as to expose the fold of mesentery corresponding to a loop of intestine. The mesentery is stretched evenly over the edge of a small capsule, and if there be fluid, blood, or other impurity adhering to its surface, it is washed with a very gentle stream of distilled water. There is then poured over it the solution of nitrate of silver until it becomes milky. It is then washed again with distilled water, and cut away carefully a little outside the edge of the capsule. The membrane is then floated off the capsule into distilled water or spirit, and exposed to the light until it becomes brown.

In the case of the frog, the animal is decapitated, the spinal cord destroyed, and the bleeding from the divided vessels of the neck encouraged by gentle pressure on the sternum. When all hæmorrhage has ceased, the skin along the front of the body is divided in the middle line, the sternum removed, and the abdomen laid open to the pubis. The intestine is turned over to one side, and there is seen at the back of the abdomen a fine transparent membrane covering over the front of the spinal column with the aorta and sympathetic nerves, and separating from the abdomen a large space, the *cisterna lymphatica magna*. The membrane is called the *septum cisternæ lymphaticæ magnæ*. With fine scissors this membrane is separated along its outer edge, from the abdominal wall, from below upwards, first on one side, then on the other. The two incisions are then united below, dividing the lower part of the intestine. The flap thus formed is drawn forwards, and everything holding it to the trunk of the animal is severed by scissors, and thus the membrane, with all the viscera of the pleuro-peritonæal cavity, are removed together. The whole mass is placed in a capsule containing one-half per cent. silver solution, and moved freely about until the serous surfaces become milky; then washed in distilled water, and transferred to fresh water. While floating in this, the portions required for examination are cut off with sharp scissors, and transferred to water or spirit, and exposed to the light. The parts that should be removed are the septum on both sides, the mesentery, the mesogastrium, the pericardium, and in a female the mesentery of the oviducts. In all manipulations with serous membranes the utmost care must be taken to have the surface clean before the silver is applied, and to avoid touching, or rubbing, or any other form of mechanical violence, by which the cells are readily detached.

In order to mount as a permanent object a piece of silvered serous membrane the following method should be adopted :—

While the object is in the spirit or water in which it has undergone the action of light, a piece of the membrane of suitable size to fit under the cover-glass is cut off with sharp scissors, and transferred to a saucer containing clean water. The piece selected should be thin, and should not contain masses of fat or large blood-vessels. It should also be borne in mind that, when the membrane is fully spread on the slide, it will be much larger than it appears as it floats in a partially collapsed condition.

A clean slide is now dipped into the water in the saucer, and with a needle the piece of membrane is floated into the right position in the middle of the glass, and removed from the water on the slide in a partially spread-out condition, that is, not folded anywhere on itself. The slide is now wiped dry, and all excess of water is removed from about the object with filter-paper until the piece of membrane is no longer wet, but only sodden, in which state it adheres slightly to the glass. By a few touches of the needle applied at the edges, the membrane can now be tensely and evenly spread. If during this process it tends to become too dry, it can be kept moist by breathing on it. When it is stretched free from every crease or wrinkle a drop of glycerine is placed on a cover-glass. This is inverted, and gently applied to the object, which, if it have been brought to the right degree of dryness previously, will not collapse, or change its position on the slide. Two pieces of filter-paper are now applied at opposite edges of the cover-glass, to remove the excess of glycerine. Enough has been taken away when the cover-glass is found to adhere to the slide when touched gently with a needle. Too much glycerine must not be taken away lest air should enter ; and besides, the removal of too much glycerine favours the running in of the cement. On the other hand, if enough be not removed, the cover-glass is very apt to become displaced while the cement is being applied. After removal of the glycerine, the slide must be wiped quite dry with a handkerchief up to the edge of the cover-glass, for cement will not stick to a wet surface. For the same reason the utmost care must be taken not to allow glycerine to get on the upper surface of the cover-glass. If this accident should occur, the best course is to put the slide back into water, and remove the cover-glass under the fluid, and begin the whole process over again. If, however, the slide has been cleaned successfully, a layer of cement is painted around the edge of the cover-glass, and the preparation is complete.

Various **cements** are in use. Of these, a solution of Canada balsam in benzole is frequently employed. The balsam before solution should be heated for some time on a sand bath, so as to drive off all the turpentine. On cooling, the balsam is hard and brittle. The solution dries rapidly. An excellent white cement is made by rubbing up oxide of zinc with a small quantity of drying oil, and adding a solution of gum dammar in benzole, mixing the ingredients very thoroughly (Richardson). Gold size, Brunswick black, and many other substances are used for sealing up preparations, and cements of all colours may be bought, and serve to increase the beauty of microscopic preparations.

In all cases the cement should be applied with a fine camel's-hair brush, with a light and steady hand. A thin layer only should be put on first, and when this is dry a second or third coat may be added. The cement should extend about one-eighth of an inch over the edge of the cover-glass, and about the same distance about it on the slide.

If round cover-glasses are used, the cementing is best effected by means of a whirling table. This is a circular disc of flat metal, which rotates in a horizontal plane. The object is fixed on the disc by clips, so that the centre of the cover-glass is immediately over the centre of rotation. Then, while the disc is made to rotate, the brush is applied to the edge of the cover-glass, which is carried round, and receives an even layer of the cement.

Instead of glycerine, Farrant's solution may be used as a mounting fluid. It consists of a thick solution of gum, to which are added some glycerine and arsenious acid. It is employed in the same way as glycerine, but the preparation is not sealed up for a day or two. By this time the gum has hardened around the edge of the preparation, and there is no risk of the cover-glass moving, or of the cement running in.

In the preparation mounted according to these directions, observe the fine black lines enclosing spaces of variable form and size. The lines are the cementing substance; the clear spaces are the **endothelial cells.** In general no nuclei are visible, but sometimes, when the silver has acted very strongly, the cells are stained brown, and the nucleus appears as a clear unstained round or oval spot. After the silvering, however, the nuclei of the cells may be stained in logwood or picrocarmine. The membrane is then washed and mounted as above.

Observe the stomata, with their small bounding cells, and larger flat cells radially placed, particularly well seen on the abdominal surface of the *septum cisternæ lymphaticæ.*

Preparations should be examined of the silvered omentum of the dog or guinea-pig, or of the mediastinal pleura of the cat or dog. These membranes are not continuous, but form a very open network. The endothelial cells can be seen lapping round the trabeculæ of the network, and if the nuclei are stained they can frequently be seen in profile, as spindle-shaped bodies lying on the sides of the trabeculæ. In these fenestrated membranes patches of germinating endothelium will often be met.

When examining thin membranes, covered on both surfaces by endothelium, either surface may be brought into view by altering the focus. Such an appearance must not be taken as indicating two layers of endothelial cells lying immediately one over the other. The two layers of cells are really separated by the thickness of the membrane.

Ciliated endothelium appears in silver preparations as irregular tracts or patches of cells smaller than those about them, and covered with minute points, which are the ends of the cilia. To see the cilia in motion, portions of the fresh membrane must be carefully spread on the slide in a drop of salt solution, and the cover-glass must be prevented from pressing on the cells by supporting it at the edges with little fragments of thin glass. The wavy motion of the cilia will then be seen.

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CHAPTER V.

CONNECTIVE TISSUES.

THE tissues belonging to this great class, although differing in many respects from one another, yet present so many points of agreement as to make their grouping very natural. They all arise from the middle germinal layer of the embryo. They replace one another not only in the corresponding parts of different animals, but also in the same animal at different stages of its development. They all consist of cells and of intercellular substance, and the intercellular substance is the most prominent and characteristic constituent of each tissue. These connective tissues are further of interest from their close relationship with the lymphatic system, and from the fact that it is in them that many morbid processes arise and run their course.

The following is a **list of the connective tissues**:—1. Mucous tissue; 2. White fibrous and yellow elastic tissues, which are always mixed, although in very variable proportions, so as really to constitute but one tissue. When 'connective tissue' is commonly spoken of it means this compound tissue. 3. Adipose tissue; 4. Cartilage, of which there are several varieties; 5. Bone; 6. Dentine; 7. The tissue of the cornea; 8. Retiform, or adenoid tissue; 9. Neuroglia, or the connective tissue of the nerve-centres and retina.

Dentine occurs only in the teeth, and will be described with these organs. The cornea will be described with the eye; adenoid tissue with the lymphatic glands; and neuroglia with the nerve-centres. The other connective tissues will be described in this place.

1. **Mucous Tissue**.—This is, in the case of the higher animals, essentially a fœtal tissue, occurring during early life in those parts where fatty and connective tissue exist subsequently. In the mature animal it exists only in the vitreous humour of the eye, and here in a modified form. In some of the lower animals, however, it persists, and in men it is not unfrequently met with as a pathological product in the class of tumours known as myxomata.

Mucous tissue consists, in its purest form, of spindle-shaped and stellate cells, which anastomose and form a network. The meshes of this are occupied by a fluid intercellular substance, which is of a stringy, viscid consistence, and contains a large quantity of mucin, shown by its giving a precipitate in acetic acid, not soluble in excess of the reagent. Lying in this fluid are a variable number of round cells, which perform amœboid movements.

The tissue is not easy to obtain in this form, for at a very early period fibres appear between the cells, and, multiplying more and more, take the place of the mucinous fluid, the characters of the tissue changing to those of ordinary connective tissue.

In the young embryo the Whartonian jelly of the umbilical cord is almost pure mucous tissue, while at birth it has quite lost this character, and consists of fibrous bundles, with large flat cells lying on their surface, although the fluid which fills the interstices of these bundles still contains much mucin.

2. **Connective Tissue**.—This consists of two tissues, which are so constantly met with in connexion, that they may be considered together. These are white fibrous and yellow elastic tissues. Connective tissue is the most widely distributed tissue in the body. It forms the skin and serous membranes, many mu-

cous membranes, fasciæ, ligaments, tendons. It forms the areolar tissue which lies under the skin, between muscles and around blood-vessels and nerves. It forms, further, the capsules of the viscera, and enters into their interior, binding the parts together, while it at the same time separates them. In all these parts the tissue is the same, although there is considerable variety in the arrangement of the elements.

White fibrous tissue consists of cells and of intercellular substance. The latter is fibrous. The fibres are extremely fine, of soft silky appearance, and comparatively low refractive power. They sometimes occur singly, felted together more or less closely, but generally are associated in bundles, of which all the fibres run parallel to one another, not branching or anastomosing. These bundles of fibres commonly are seen to pursue an undulating course through the field of the microscope. When treated with acetic acid the fibres of white fibrous tissue swell up, and become transparent, assuming a glassy homogeneous appearance. They yield gelatine on being boiled.

The fibres of **yellow elastic tissue** never run in bundles. They are of all degrees of fineness, from threads scarcely visible with a high power to very coarse fibres. They almost always branch and anastomose very freely, so as to form a more or less open network. They have a peculiarly hard outline, owing to their high refractive power. They break with a sharp fracture, and the broken ends have a tendency to curl up. They are completely unacted on by acetic acid, and by this reagent they may be always readily detected among the bundles of white fibrous tissue. When boiled, they yield not gelatine, but elastine.

White fibrous and yellow elastic tissue occur mixed together in very variable proportions. In some few places, as the ligamentum nuchæ, the ligamenta subflava, and some of the ligaments of the larynx, the yellow elastic tissue preponderates, but in most places as tendons, fasciæ, &c.: the white fibrous tissue exists in by far the larger proportion.

The **cells of connective tissue** are for the most part flattened bodies, each containing a nucleus, about which the cell is thickened, but at the margins runs out into an exceedingly thin film. From the surfaces of these cells there frequently project wing-like processes. When seen from their edges, the cells are spindle-shaped or stellate. They lie usually on the outside of the bundles of white fibrous tissue. Besides these flat cells, whose positions are unalterable, there are found others, which resemble the white corpuscles of the blood, and which do not remain fixed in one part of the tissue, but move about from place to place, and hence are called **wandering cells**. Connective tissue cells of a third variety occur. They are large, and more coarsely granular than the wandering cells, and of variable shape. They are found in many places, and lie chiefly close along by the sides of the blood-vessels. They have been called **plasma cells** (Waldeyer).* In some parts the connective tissue cells contain pigment. In frogs such **pigment cells** are to be found everywhere. They are usually branched, and form very beautiful objects. In the higher animals they are not so common, but occur in the choroid coat of the eye, the pia mater of the medulla oblongata, and a few other places.

Connective tissue will now be described as it occurs—1, in tendon; 2, in a serous membrane; 3, in the so-called formless connective tissue or areolar tissue.

In **tendon** the fibres of white fibrous tissue all run in bundles, and the bundles in general run parallel to one another. The bundles are cylindrical, or may have their sides slightly flattened by mutual pressure. The cells are flat, and lie with great regularity in rows on the outside of the bundles. Each cell is somewhat quadrilateral in shape. Extending down the middle of it, parallel to the direction of the fibrous bundles, is a thickened part, at one end of which is contained the round or oval nucleus. Extending laterally, the cell thins out very much, and terminates by a jagged border. The cell is lapped around the bundle, but is not large enough to reach the whole way round. The cells of each row are separated from one another by very slight intervals, which are often disposed obliquely to the direction of the fibres. The nucleus of the cell lies, as stated, near the edge, and as a rule the nuclei of every pair of cells lie close to one another, one at each side of the separating interval, so that there are alternately an interval with two nuclei and one without any. From the convex surface of the cells extend one or more ridges, which fit in between neighbouring bundles. It is probable that the rows of cells do not lie in immediate apposition with the bundles of fibres, but that each of these is enclosed in a homogeneous sheath, and that the cells lie in spaces in a soft cementing substance outside this. There is only a small quantity of elastic tissue in tendons. It occurs as fine threads among the white fibres in the bundles. If the tendon is small it contains no vessels; if large, vessels extend into it, accompanied by more or less formless connective tissue. The whole tendon is enclosed in a firm sheath, which is covered by a regular endothelium.

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* It is probable that different species of cells have been grouped together under the term plasma cells. One of these is distinguished by the intense colour which the granules assume in certain aniline dyes (Ehrlich).

In some **membranes** the fibrous bundles are arranged with great regularity, and cross each other at right angles. In such cases the cells are seen to have ridges not only in one direction, as in tendon, but in two planes at right angles to one another. This shows clearly that the ridges are determined by the fibrous bundles, which mould by their pressure the soft protoplasm of the cells (Ranvier).

In the serous membranes, however, the bundles are not so regularly placed.

Some membranes are fenestrated, or consist of a fibrous network, with wide meshes. This is the case in the human omentum and in that of many animals. The bands which form the network are of very different breadth. The narrower consist of a single bundle of fibres; the thicker of several bundles lying together, and enclose also blood-vessels, lymphatics, and fat. The entire network is covered by endothelial cells, and except this endothelial sheath, the smaller bands possess no cells. In the larger bands, however, besides the general coating of endothelium, there are flat cells lying between the fibrous bundles, and appearing generally as spindle-shaped bodies, since they are seen in profile.

In a continuous membrane, such as the mesentery, in which there are no holes, the bundles are very closely felted together, and generally appear running a wavy course in all directions, and interlacing with each other. Filling up the intervals between the bundles is a homogeneous material, probably an albuminous cementing substance, but capable of distension when the membrane is forcibly stretched. The elastic tissue exists in variable quantity, as a network of very fine threads, which branch and anastomose freely. In preparations treated with acetic acid or dilute alkali, or suitably stained, they are very distinct; but even in fresh preparations they are readily distinguished by their hard outline, their rectilinear course, and their branching, from the soft silky-looking bundles of white fibrous tissue. It will generally be found, on careful focussing, that the elastic network lies nearer to the surface than the bulk of the white fibrous tissue.

The membrane is covered with endothelium; but, besides this, other cells are found—some which lie flat on the surface of the membrane under the endothelium, and others which lie between the bundles and partially embrace them. The cells are, however, not so numerous as in tendon, nor have they so regular an arrangement.

The **formless connective tissue** exists in largest quantity beneath the skin, as the subcutaneous areolar tissue. It consists of bundles of white fibrous tissue, which run in all directions, and interlace with one another. The spaces, or areolæ between the bundles, are capable of considerable distension, and communicate freely with one another, as is familiarly shown by the extension over the body of an emphysema when the lung has been wounded, or by the movements of a dropsical effusion, so as to occupy always the most dependent parts.

When the bundles are treated with dilute acetic acid they swell, and are seen to be constricted at intervals by threads, which either surround them annularly, or are twisted about them in a spiral. It is probable that each bundle is enclosed in a sheath, which is strengthened by these thread-like structures. There is some doubt as to the nature of the constricting threads. They were at first described as elastic tissue, and are commonly still so considered ; but the fact that they stain red in picrocarmine is against this view, for elastic tissue stains yellow in this reagent (Ranvier). They are very well seen on the bundles of the subarachnoid tissue from the base of the brain, and here it can sometimes be distinctly seen that the bundle is embraced by a flat cell at the point of constriction.

Elastic tissue is abundant in formless connective tissue, and occurs in the shape of fine threads, which usually pursue a very tortuous course in the preparations, being twisted into loops, spirals, or convolutions. In the tissue, before removal, however, they run a straight course, and only twist when they are divided, just as an India-rubber thread does when it is snapped. They branch more or less freely, and anastomose. With a little care they may be seen in fresh tissue, but they are more distinct after the action of acetic acid or potash. The cells here also are flat endothelial plates, and lie on the outsides of the bundles. Several cells frequently cohere by their edges, so as to form membranous structures, which are stretched between the fibrous bundles (Key and Retzius). They are very readily detached from their natural position, and hence in preparations are usually found lying isolated. In consequence also of their extreme thinness and delicacy they are very apt to undergo injury, and to appear as spindle-shaped or irregularly shrivelled bodies, instead of presenting their normal form.

Amœboid wandering cells are met with in variable number, and in places plasma cells.

III. **Adipose tissue** consists of cells which lie close together, and each of which contains one or more drops of fat. In the fully developed adipose cells the fat drop is so large that the whole cell is swollen to a spherical mass, the nucleus flattened and pushed to one side, and the protoplasm distended, so as to form a thin coating of the globule of fat. These fat cells lie in groups, each group being separated from its neighbours by a small quantity of connective tissue. It is these little groups of fat cells which give to adipose tissue its granular appearance when examined with the naked eye or with a lens. Adipose tissue is very abundantly supplied with blood-vessels, almost every fat cell being surrounded by a capillary loop. In this it contrasts much with connective tissue, which is very poor in vessels.

Adipose tissue is continually undergoing formation and atrophy in the bodies even of adult animals. It is still a question whether the fat is formed in the interior of ordinary flat connective tissue cells, or whether there are certain special cells whose destiny it is to become adipose tissue, and which can alone undergo this development. The latter view is, however, by far the more probable. The cells which subsequently become those of adipose tissue appear at first as polygonal, coarsely granular bodies, lying generally in masses in the neighbourhood of blood-vessels. In the interior of each cell there appears at first one or often several droplets of fat: these enlarge, and finally coalesce. At first the protoplasm and nucleus can be easily seen, but at last, as the fat drop increases, these are pushed aside, and can be seen only with difficulty, and when the cell lies in a suitable position. When fat is undergoing atrophy the oil drops in the cells diminish in size, and the space around them is occupied by a serous fluid. The fat assumes a yellow colour, and the single fat drop often divides into several smaller ones. At the same time a remarkable change is frequently observed in the nucleus, which proliferates until there are sometimes a dozen or more nuclei in a single cell. What the fate of these nuclei is, is not clear. There is some reason to believe that each of them surrounds itself with a layer of protoplasm, and, being set free, becomes an independent cell.

In young animals the characteristic arrangement of blood-vessels exists in the masses of cells about to form adipose tissue before any fat has been formed in the interior of the cells.

Mucous Tissue:

1. A small fragment of subcutaneous tissue from a young fœtus of man or other mammal is separated very gently in a drop of salt solution, covered and examined. If a permanent preparation be desired, the tissue should be torn up in picrocarmine, covered, and placed in the moist chamber. In a short time the staining will be effected, when glycerine may be allowed to run in under the cover-glass, and take the place of the picrocarmine. Observe the fusiform and stellate anastomosing cells; the homogeneous intercellular substance, in which round cells like white blood corpuscles, and some fibres, may be seen. If the preparation in salt solution be irrigated with acetic acid it will be seen to grow white and opaque, and the microscope will show a granular precipitate of mucin in the intercellular fluid.

2. The umbilical cord should, if possible, be examined from fœtuses of different ages. It should be hardened in Müller's fluid and alcohol. Sections, best made by freezing, should be stained in picrocarmine or logwood, and examined in glycerine. If necessary they may be torn up. As the age of the fœtus increases, the cells will be seen to be less fusiform and

plump, and to become flattened scales, which, however, still anastomose with one another by processes.

Formless Connective Tissue:

3. Turn back a flap of skin from a recently killed rabbit. In this animal the subcutaneous tissue is free from fat. From the deep surface of the skin pinch up with forceps a fragment of the areolar tissue, and cut it off with scissors. Place the fragment dry on the slide, and spread it out with needles in as thin a layer as possible. There will be no difficulty in this, as the morsel of tissue will adhere to the glass; but it must not be allowed to get altogether dry; and in order to prevent this it should be breathed on frequently. When it is spread out, place a small drop of salt solution in the centre of the cover-glass, and apply this quickly to the object, so as to prevent the tissue from contracting. Examine with a high power. The bundles of white fibrous tissue will be seen running in different directions over the field. Observe their wavy course, their fibrous structure, and their soft silky appearance. Try to distinguish the elastic threads, fine branching filaments, of hard outline, often bent or curled at their broken ends. There may be seen, here and there, an oval nucleus of a connective tissue cell, and by careful observation the protoplasm may be seen about it.

Irrigate this preparation first with magenta, then with water. If the magenta runs round the preparation, and stains only its edge, it may be necessary to raise the cover-glass. This should be done cautiously with the point of a needle, and care must be taken that the fragment of tissue does not contract. The magenta will stain the nuclei, and make them and the cells more distinct. It will stain the elastic tissue deeply; the white fibrous bundles much less.

4. Make a fresh preparation of the subcutaneous tissue as in the last exercise. Irrigate with one per cent. acetic acid. The white fibrous bundles will swell up and become transparent, and all trace of fibrillation will disappear; the elastic tissue will be unacted on; the nuclei of the cells will become more distinct. As the acid acts at first only at the margins of the preparation, it will be possible to see, often in the same field, the tissue before and after the action of the reagent. Try to see constrictions on the white fibrous bundles.

5. For more accurate study of this tissue the method of *artificial œdema* should be employed. By this the bundles are separated from one another while their continuity is still unbroken. Turn down a flap of skin; insert obliquely into the subcutaneous tissue on its deep surface the cannula of a hypodermic syringe, and slowly inject salt solution. A hemispherical projection forms about the end of the cannula, and, owing to the condensation of the tissue around it, the fluid does not spread at once diffusely. With flat scissors cut off the top of the projection, and its interior will be seen to consist of a gelatinous mass,

composed of the bundles of the tissue with the injected fluid lying between them. Cut off a small piece of this jelly with sharp scissors, and place it on a slide, and cover at once. Both slide and cover-glass must be ready, as it is essential that the proceeding be carried out rapidly, before the elements of the tissue run together. In this preparation the appearances will be as in 3, but the elements, particularly the cells, will be much better preserved. Try to see the cells, some in face, appearing as flat bodies; others on edge, as spindle-shaped figures. In such a preparation picrocarmine may be substituted for the salt solution, by drawing out the latter with filter-paper at one edge of the cover-glass, while from the opposite the colouring fluid is allowed to run in. When the staining is effected, a drop of glycerine containing one per cent. formic acid may be substituted for the picrocarmine, and the preparation may be sealed.

Or the artificial œdema may be made with picrocarmine, instead of with salt solution. Or with some fluid which sets the tissue at the same time it separates its parts, as half per cent. solution of osmic acid. In this latter case so much haste in making the preparation is not necessary as when salt solution is used, since the tissue, after the action of osmic acid, has lost its retractility. The tissue may be mounted in a drop of picrocarmine, and be placed in the moist chamber for twenty-four hours, when glycerine may be substituted for the picrocarmine, and the preparation sealed up.

6. Take a small fragment of the subarachnoid tissue from the middle subarachnoid space at the base of the human brain. Place it in a watch-glass with picrocarmine for some hours until it is well stained; then gently separate on a slide some of the fibrous bundles, and place on them a drop of one per cent. acetic acid; remove the acid with filter-paper, and mount in glycerine. Observe the annular and spiral constrictions, with the swelling of the, now homogeneous, bundles between. Try to find places where cells lie on the outside of the bundles, and embrace them by their processes.

Tendon:

7. In the tails of rats, and, still better, of mice, tendons exist in large numbers and of great length, and so thin that they may be examined without any preparation.

From a recently killed mouse cut off the tail and skin it; pinch off the last joint with a forceps, and gently draw it away from the remainder of the tail. Adhering to the separated joint there will come away a number of extremely fine white shining threads, as long as the tail itself. Each of these threads is a complete tendon. By pinching off each joint in succession any required quantity of these threads may be got. The nearer the joint is to the body of the animal the shorter, of course, are the tendons attached to it. The tendons, owing to their great thinness, dry very rapidly. Consequently, as each lash of tendon is withdrawn, it should be plunged into a vessel of salt solution.

Cut off half an inch or so of one of the threads, and place it on a slide; cover in salt solution, and examine. Great care must be taken that the tendon does not get twisted on its long axis, which would completely destroy its natural appearance. It is best to place the piece of tissue on the slide in a large drop of salt solution, and allow it to settle itself, and then take away the excess of fluid with filter-paper. Do not compress the preparation with the cover-glass.

Observe the fibrous structure, the fibres all running parallel, and pursuing generally a wavy course; the division into bundles of nearly equal size by dark lines, in which may be seen indications of the tendon cells, as rows of elongated granular bodies.

Irrigate with acetic acid. The bundles swell, become perfectly transparent and homogeneous, and in the interspaces there appear rows of long spindle-shaped nuclei, around each of which there may be seen the oblong granular mass of protoplasm, forming the thicker central part of the cell. A very few long and very fine elastic fibres may be noticed.

8. Take a piece of the fresh tendon from the salt solution, hold it by one end in the forceps, and dip it into dilute acetic acid until it loses its lustre, and appears glassy and transparent. The change can readily be seen with the naked eye, and requires only a few seconds for its completion. Then withdraw the tendon from the acid, wash freely in water, and place it in a watch-glass containing filtered logwood for about an hour. Then wash in water, place on the slide, and very slightly separate the bundles from one another with needles. In doing this the tendon should not be torn up, but slightly widened, and the needles should be applied to as few points as possible. Cover in a drop of glycerine, and very gently press on the cover-glass with the needle, so as somewhat more to flatten the preparation. The tendon cells are stained blue, while the fibrous bundles are unstained. The acid has, however, made them so transparent that the different rows of tendon cells can readily be seen by altering the focus. These rows of cells will present themselves, some in profile, others in face, and in all intermediate positions. On those cells which present their surface to view there may be seen one or more dark lines continued all along the row. These are the ridges which project from the convex surfaces of the cells, and fit in between neighbouring bundles. In some parts of the preparation the needle will have displaced the cells altogether from their natural position. In some of these dislocated cells the shape and structure may be very well seen; the thickened centre in which the nucleus is situated; the peripheral wing-like expansions, and the ridges projecting from the central part. This preparation may, if successful, be sealed up.

Pieces of tendon stained in picrocarmine, treated with strong acetic acid, and mounted in glycerine, also give instructive preparations.

9. Take a piece of tendon immediately it is removed from the animal, dip it into half per cent. nitrate of silver solution for two or three minutes, wash in distilled water, and expose to the light. When brown, mount in glycerine. The surface of the tendon will be seen covered by a large and regular endothelium, similar to that seen on the serous membranes.

10. Take a piece of fresh tendon, dip it into nitrate of silver for a few minutes, then remove it to distilled water; hold it by one end with forceps, and with a camel's-hair pencil brush it strongly in length, and return it to the silver for five minutes more; then wash it in water, and expose it to the light, and when brown mount it in glycerine. The endothelium will have been removed by the brushing, but there will be seen, on a brown ground, rows of oblong or irregular figures unstained, and which communicate with one another by fine clear lines. The white figures are the spaces in which the rows of tendon cells lie. We shall return to this when speaking of the origin of the lymphatics. 9 and 10 may be mounted permanently.

11. In order to get a cross section of tendon it is best to cut the entire tail. The appearances are most distinct after staining in chloride of gold. Pieces of the fresh tail about half an inch long, and skinned, are placed in half per cent. solution of chloride of gold for an hour, then washed in distilled water, and exposed to the light in water slightly acidulated with acetic acid, until they become of a purple colour. They are then placed for some hours in a fluid composed of chromic acid, one-half: nitric acid, one; water, one hundred, until the bones are decalcified, which is accomplished when a needle can be passed through the bone without meeting any grit. They are then washed free of acid, and placed in alcohol. Cross sections are made by the method which will be described in the next chapter, and mounted in glycerine. Surrounding the bone the tendons are seen, each enclosed in its sheath. Each tendon is marked by a number of stellate anastomosing figures of a purple colour. These are the sheaths of the tendon bundles, which send processes, partly lamellar, partly thread-like, into the interior of the bundles. In the thickened parts of these figures, that is, between the bundles, there may sometimes be seen the nuclei of the tendon cells, which lie on the outside of the sheaths. The bone, nerves, muscles, and other structures, which make such sections very instructive, may be neglected for the present. Long sections of the tail may be made also. The tendons are now cut in length, and the rows of tendon cells are seen. They have a purple colour, while their nuclei are unstained.

12. In order to isolate the fibres of which the connective tissue bundle is composed, it is necessary to employ some chemical reagent which will dissolve the cementing substance

which holds the fibres together. A tendon from the tail of a mouse is placed for twenty-four hours in a saturated watery solution of picric acid, and then torn up with needles on the slide. The fibres are so exceedingly fine that they are difficult to see when immersed in fluid. They are, therefore, best examined after they have dried on the slide. The fibres will have lost their natural parallel arrangement, and form a confused tangle, in which one single fibre cannot be followed for any great length. The cover-glass may be fastened down with narrow strips of gummed paper, and the preparation thus made permanent.

In the examination of larger tendons, such as those of man, it is necessary to make sections, which should be either longitudinal or transverse. The tendon should be hardened for some days in alcohol, or dried, in order to give it a fit consistence for cutting. The sections are moistened in water, in which they may be examined; or stained in logwood or picrocarmine, and mounted in glycerine. On transverse sections, the tendon is seen to be surrounded by loose connective tissue, which sends into the interior septa enclosing blood-vessels. The interspaces of the tendon bundles, and which contain the tendon cells, will be seen as stellate figures.

On longitudinal sections, the parallel tendon bundles will be seen, separated by the rows of apparently spindle-shaped cells.

Serous Membrane:

13. From the abdomen of a recently-killed rabbit, a portion of the mesentery, preferably that of the colon or rectum, is snipped off with scissors, and placed on a dry slide. It is carefully spread with needles, while it is prevented from drying completely by being breathed on from time to time. A drop of salt solution is placed on the cover-glass, and this is laid on the preparation, which should remain fully extended. This extension is ensured by causing the drop of salt solution to touch first the centre of the piece of membrane, and to come into contact with its edges only at the moment the cover-glass falls on it.

The membrane will be seen to consist of a groundwork of wavy bundles of fibrous tissue, so closely placed and interlaced in such an intricate manner as to make the appearance at first sight somewhat confusing.

On altering the focus slightly, an abundant elastic network will come into view. The elastic fibres will readily be recognised by their hard outlines, and free branching and anastomoses.

14. Remove the cover-glass from the last preparation, and take away the excess of salt solution, and dry the slide about the piece of membrane which will now have retracted; spread the tissue again as at first, and replace the cover-glass, having placed on it a drop of dilute acetic acid.

The wavy bundles will now be very transparent or indistinct, or have altogether disappeared. The elastic network will be very evident, and a number of nuclei will be seen. Some of these are oval, and lie on the surfaces of the membrane, as can be determined by altering the focus and noticing that they are the last objects seen before every part of the preparation becomes indistinct by passing out of focus. These are the nuclei of the endothelium. Besides these, there are others—some oval, others spindle-shaped—and which lie deeper than the endothelium, on the surfaces of, or between, the connective tissue bundles. These are the nuclei of connective tissue cells. They are much more numerous in some parts of the peritonæum than in others.

15. Permanent preparations of the serous membrane may be made by staining the piece first in logwood, not too deeply, then washing in water, and staining for a few minutes in a dilute watery solution of eosin, washing again, spreading carefully on the slide, and covering in glycerine. The connective tissue bundles are stained light red, the elastic fibres intensely red, and the nuclei of all kinds blue. Some practice will be necessary to succeed with this preparation.

16. The omentum of a dog, cat, or guinea-pig, in which animals, as in man, this membrane is fenestrated, is placed for some days in two per cent. solution of bichromate of ammonia; then carefully washed by being placed in water, until it ceases to give up colouring matter. Small pieces are stained in logwood, washed in water, floated on to the slide, accurately spread, and covered in glycerine. A beautiful network will be seen. On the surface of the bands forming this there are present nuclei stained blue. Some seen in face, and of oval shape; others in profile, lying on the sides of the bands, and appearing spindle-shaped. These are the endothelial nuclei. In the broader bands are seen nuclei of connective tissue cells which lie between the bundles, and are more deeply situated than the endothelium.

Far more beautiful preparations may be got by staining the omentum, first in silver nitrate and then in picrocarmine or logwood, and mounting in glycerine in the usual way. The outlines of the endothelial cells, as well as the nuclei, can then be seen. It will be noticed how the smaller bands are entirely wrapped round by a single cell, a fine black line, generally along the margin, marking where the edges of the cell meet. On the larger bands a regularly-shaped endothelium will be seen, and under this the nuclei of the connective tissue cells. These preparations may be preserved permanently.

17. It is of some interest to see the course of the individual bundles in such a membrane as the omentum, and to study their relations to the spaces in the network. This is difficult to do when the membrane lies in fluid, owing to the great trans-

parency of the tissue; but if a piece of the fresh membrane be spread on a slide and allowed to dry completely, and examined in this dry condition, the course of the bundles is readily made out. They are seen to interlace in all directions, and, separating from one another, to leave the spaces between them. Each space is not bounded by one bundle which encircles it, but many bundles, passing in different directions, contribute to form the boundary (Ranvier). Such a preparation may, if desired, be preserved by covering it without adding any fluid, and fixing the cover-glass by strips of gummed paper.

Adipose Tissue:

18. In the larger bands of the omentum and in the mesentery adipose tissue will be found in variable quantity. The fat cells lie along by the sides of the blood-vessels. They can be very beautifully seen in silver preparations, when the nuclei have been stained by logwood or picrocarmine. Each cell is of spherical shape, and consists, for the most part, of a large globule of fat, which looks bright in the centre and dark at the margin, owing to its high refractive power. Around the fat is a thin layer of protoplasm appearing as a fine line. At one side this is thickened, and here lies the flattened nucleus, appearing spindle-shaped when seen in profile.

19. Osmic acid is reduced by fat, which becomes black when treated with this reagent. A piece of mesentery containing a quantity of fat, barely perceptible to the naked eye (if the fat be in large masses the preparation is too thick for examination), is removed from the freshly-killed animal and placed for half an hour in a one per cent. osmic acid solution. The osmic acid should be placed in a watch-glass, and kept covered with a glass plate, as its vapour is extremely irritating to the mucous membranes of the eyes and nose. The membrane is then well washed in distilled water, and placed for twenty-four hours in picrocarmine, washed and mounted in glycerine. The fat cells will now be seen as black spheres. The nuclei are red.

20. A piece of adipose tissue may be torn up in a drop of salt solution. The fat cells are isolated. Many of them are ruptured, and the escaped fat floats about in the fluid, while the collapsed membrane of the cell with its nucleus may be seen.

21. The yellow gelatinous substance found under the visceral pericardium of persons who have died of phthisis or other wasting disease consists of atrophic adipose tissue. A small piece of this torn up in a drop of picrocarmine, placed in the moist chamber until stained, and then mounted in glycerine, shows the cells losing their fat, which now occupies only a small part of the space it previously filled, a serous fluid having taken its place. Some of the cells will be seen to contain large numbers of nuclei.

When adipose tissue has lain for some time in alcohol, and often in preparations that have been mounted in glycerine, the fat crystallizes, and radiating masses of acicular crystals can be seen in the interior of the cells.

Elastic Tissue:

22. Tear up in water a small portion of the ligamentum nuchæ of an ox. Observe the highly refracting, homogeneous elastic fibres, much coarser than any seen in previous preparations. They branch and anastomose freely, break with a sharp fracture, and the broken ends curl, owing to their elasticity. Between them is found a small quantity of wavy connective tissue.

Add acetic acid, or dilute potash solution, and see that it is without effect on the elastic fibres.

IV. Cartilage:

This tissue consists, like the other members of the connective tissue series, of cells and of intercellular substance; and it is to the intercellular substance that its most remarkable properties are due.

According to the nature of this intercellular substance three varieties of Cartilage are described: 1, Hyaline; 2, Elastic, or reticular; 3, Connective-tissue, or fibrous.

1. **Hyaline cartilage** is very widely distributed in the body. The cartilages of incrustation on the articular ends of the bones; the cartilages of the synchondroses; the costal cartilages; the cartilages of the larynx (with those exceptions which will be afterwards made) and bronchi; the cartilages of the nose and part of the Eustachian tube; and in the fœtus, almost the entire skeleton, are formed of hyaline cartilage. This tissue is firm, elastic, flexible within certain limits, beyond which it breaks with a sharp fracture; of a bluish-white, semi-transparent appearance, and contains very few blood-vessels.

Examined in thin sections, it is seen to consist of cells set in an intercellular substance, which appears structureless, or very finely granular. The cells differ in size, shape, and arrangement in different cartilages. They may be flattened, round, oval, or angular. In some animals they are branched, but in the human subject this variety is met with only in pathologicallyformed cartilage. As a general rule, when the cartilage has a free surface, the cells next this are flattened, and lie parallel to the surface of the cartilage. In perpendicular sections they consequently appear as linear bodies. This is the case in the articular cartilages, those of the larynx and bronchi, costal cartilages, &c. When cartilage abuts on bone the cartilage cells lying next to the latter are arranged in long rows which stand perpendicularly to the line of junction of the two tissues. This is the case in the articular cartilages and costal cartilages. The cartilage cells very commonly are not distributed with perfect evenness, but occur in little groups; and the shape and arrangement of the cells in these groups are such as to suggest that the entire group has arisen by division of a single cell.

The cartilage cells are composed of a finely granular protoplasma, containing one or two roundish nuclei. Embedded in the protoplasm it is not uncommon to find fat drops, which stain black with osmic acid; or glycogen, which becomes dark reddish brown with iodine.

In the natural condition the cartilage-cell completely fills the cavity in the intercellular substance; but under various influences the protoplasm shrinks, and leaves a considerable space about it.

The **intercellular substance** is, as stated, apparently homogeneous, and either structureless or very finely granular. There is reason to believe, however, that this homogeneity is only apparent, and that really, as in the other connective tissues, the intercellular substance of cartilage is fibrous, the fibres being held together and rendered invisible by a large quantity of highly refractive cement-substance. When cartilage is treated with reagents which dissolve cement-substances, a fibrous structure becomes apparent; and, in pathological conditions, the fibrous change of the intercellular substance is very common.

Surrounding each cell is a narrow border, which differs in refractive power from the rest of the intercellular substance: this is called the **cartilage capsule**. It is common to find in one capsule several cells, each surrounded by a secondary capsule of its own; and even three or more generations of capsules may be found enclosed, one within the other.

It may frequently be seen that the intercellular substance about the cells or groups of cells differs from that which lies mid-way between, or most distant from these. The former parts are more structureless, the latter finely granular. The former are more recently formed, the latter older. Staining fluids of different kinds make these differences more distinct; and the cartilage is thus mapped out into compartments, each consisting of one or more cells, surrounded by recent intercellular substance, and separated from one another by the older material. These compartments are called **cell territories.**

On careful examination it can sometimes be seen that the cartilage capsules are perforated by minute radial pores. And it has been stated that the intercellular substance is traversed by fine canals, by which the cell spaces communicate with one another, and that it is by these channels that the lymph circulates through the tissue. This statement, however, requires further confirmation.

Where cartilage occurs in small masses it contains no blood-vessels; but in larger pieces canals exist which contain, besides vessels, some loose connective tissue with numerous cells.

Except in the interior of the joints, the free surfaces of cartilages are covered by a membrane composed of fibrous and elastic tissue. This is the **perichondrium,** and its deeper layers are attached to the cartilage, the fibrous bundles becoming continuous with the hyaline intercellular substance.

Cartilage when boiled does not yield gelatine, as do the other connective tissues, but chondrine.

In the cartilages of old persons it is very common for the intercellular substance to calcify. The deposition of the earthy material begins immediately around the cells, the cartilage assumes an amber yellow colour, and cavities containing gelatinous fluid are formed in the calcified part. The intercellular substance is in places composed of stiff unbranching fibres lying side by side; and the cells in the neighbourhood show evident signs of proliferation.

2. **Reticular or Elastic Cartilage.**—This tissue occurs in the external ear, the epiglottis, part of the arytenoid cartilage, and the cartilages of Wrisberg and Santorini, and in part of the cartilaginous portion of the Eustachian tube. It is of a yellowish colour, and much more opaque than hyaline cartilage, and being much more flexible, does not snap on being bent, as this tissue does. Reticular cartilage consists of cells, singly or in groups, and becoming flattened where they approach the surface. Each cell is surrounded by its capsule, and lies in a hyaline material precisely similar to that forming the intercellular substance of hyaline cartilage; but this structureless substance is traversed by a **net-work of elastic fibres,** and it is to these that the peculiarities of the tissue are mainly due. This net-work may consist of coarse fibres, forming a comparatively open reticulum, and consequently leaving in its meshes a great deal of hyaline material; or the net-work may be so fine and close as to give a spongy or granular appearance to the tissue, and seemingly to completely displace all the hyaline material, except the capsules of the cells. All gradations between these two extremes may be met. In the arytenoid cartilage, where both hyaline and reticular cartilage occur, the transition from one tissue to the other is seen to be caused by the appearance and gradual increase of elastic, branching, and anastomosing threads in the hyaline basis substance. The earliest appearance of the elastic material is in the shape of granules arranged around the cells. In

general, near the surface the elastic net-work is less abundant than in the deeper parts.

The perichondrium here is similar to that in hyaline cartilage. Blood-vessels, when they occur, are surrounded by connective tissue. Reticular cartilage does not calcify.

3. **Connective-tissue or Fibrous Cartilage.**—This occurs in the cartilaginous edges of the sockets for the humerus, femur, &c., in the intra-articular cartilages of the knee, jaw, sternum, &c., in the symphyses and intervertebral discs. It is a white, opaque, very tough, elastic, and flexible tissue. It consists of oval or round cells enclosed in capsules, and set in an intercellular substance consisting of bundles of white fibrous tissue. It differs from this latter tissue, in that the cells are not flat, and that they are enclosed in capsules. The transitions from one tissue to the other can be well seen in the intervertebral discs, where next to the bone is a thin layer of hyaline cartilage: on this follows fibrous cartilage, which gradually passes into pure fibrous tissue. Fibrous cartilage has no proper perichondrium. It contains few vessels. It is not prone to calcify.

There are some few parts in the lower animals, where cartilage exists in such thin lamellæ that it can be examined without being cut, but as a general rule it is necessary to make sections. Cartilage is of such a consistence as to require no previous hardening; it is firm enough to be held in the hand without being crushed; and it is transparent, so that even thick sections can be seen through. For these reasons it is an easy object on which to practice section cutting.

Cartilage is best examined fresh, either without the addition of any fluid beyond that which is contained in the tissue itself, or in serum. In these cases the cells will be seen in their natural condition, and completely filling the cavities in the intercellular substance. If, however, water be added, the retraction of the cells is not slow to occur. Many hardening reagents cause the cells to become distorted, but there are some which, while they harden and fix the cells, do so without causing them to shrink. Such is picric acid in saturated watery solution (Ranvier.) A small piece of hyaline cartilage is placed in this for 24 to 48 hours, washed in water, and preserved in spirit until required for section. The following cartilages should be examined:—Head of femur of frog; costal cartilage of rabbit or other small animal; tracheal ring of cow; cartilage of incrustation on articular end of long bone of dog, cat, or rabbit; costal cartilage of old human subject.

To cut the cartilage, the piece should be wedged into a split in a piece of soft cork. This will enable the object to be held conveniently. The sections should be made with a razor. The blade and the handle should form one line. The blade should be held horizontally with the edge turned towards the operator. The upper surface of the blade should always be well wetted with spirit, so that the sections as cut may not adhere to the razor. A saucer of spirit should be at hand, and the razor dipped into it between each cut. The saucer should lie under the hand so as to catch the drops of spirit that may fall from the razor. If the tissue be fresh, the razor should not be wetted. The razor should be held lightly, but firmly, between the fingers and thumb, not in the clenched fist. It should be entered at the heel, and drawn through the tissue towards the free end, the movements being made from the shoulder. It is not necessary at first to make very large sections, but great attention should be paid to having them thin and even. The sections as they are made should be removed from the razor with a camel's hair brush and placed in a watch-glass containing some clean spirit. When several sections have been cut, the thinnest should be selected for examination. The sections should be mounted in glycerine, diluted with an equal part of water. The appearances are seen very well without staining; but the preparations are prettier if they are coloured.

1. **Head of Femur of Frog.**—If this be fresh the sections must be taken only from the most superficial part, as the intercellular substance is calcified at a short distance below the surface. If it have lain some time in picric acid the earthy matter will have been dissolved, and the section can be made without risk. It is well to test whether the decalcification is complete, by passing a fine needle through the cartilage before cutting it.

Cells large, with generally one nucleus; capsules distinct; intercellular substance homogeneous and structureless.

Sections stained in purpurine give beautiful results: the nuclei are deep red, the protoplasm scarcely stained, the intercellular substance faint red. The staining requires 24–48 hours. The sections are then washed and mounted in glycerine.

2. **Costal Cartilage.**—The section should be made at right angles to its long axis through the entire thickness of the cartilage, including the perichondrium. It may be examined in dilute glycerine, unstained, or after staining in purpurine, or picrocarmine. Intercellular substance homogeneous; cells flattened (appearing linear) near surface; more irregular and

in groups in deeper parts: perichondrium consisting of connective tissue: between the bundles may be seen the nuclei of the flat connective tissue cells.

3. **Tracheal Ring of Cow.**—Cells very irregular in shape and lying in groups; intercellular substance about groups of cells structureless and homogeneous; these cell-territories separated by broad bands of a granular substance, in which are no cells. Sections stained first slightly in logwood and then in eosin give very clear appearance. Cells and nuclei bright red; homogeneous intercellular substance blue; granular boundaries of cell-territories red, but less intense than nuclei.

Canals bounded by connective tissue, and containing vessels, may be seen in these sections.

4. **Cartilage of Incrustation** on articular end of long bone. To examine this, the bone must be decalcified by the method to be described in the next section. Sections must be made perpendicularly to the surface through the entire thickness of the cartilage and part of the subjacent bone. They are to be mounted in dilute glycerine. The cartilage consists of four layers:—1. In the most superficial the cells are flattened and appear linear in the section. 2. This is followed by a layer in which the cells are rounded and lie in groups. 3. A layer in which the cells lie in elongated rows, which are placed perpendicularly to the line of junction with the bone. It will be seen that each row of cells is enclosed in a common capsule, each cell having its own secondary capsule. This layer passes into 4, a layer in which the cells are still arranged in rows, but in which the intercellular substance is calcified. The limits between 3 and 4 are indicated in decalcified preparations by a fine line. The junction with the spongy bone is by an irregularly festooned edge.

A very interesting observation may be made on articular cartilage when it is examined by the polarising microscope. There are many substances which in their ordinary condition exert no influence on polarised light, and are said to be singly refracting or isotropic. Such is glass and the intercellular substance of cartilage. If, however, such substances be subjected to compression or strain, their molecular constitution is so altered that they become doubly refracting or anisotropic, and decompose the polarised ray into two. If under the stage of a microscope a Nicol's prism, or polariser, be placed, so as to polarise in a certain plane the light which passes through the tube of the instrument, and if above the eye-piece a second Nicol's prism, or analyser, be placed in a position corresponding to that below, the field will appear bright, because the light which passed through the lower Nicol can traverse the similarly placed upper prism. If now the upper Nicol be slowly turned round in the horizontal plane, it will be found that the field will become less and less bright; and when the rotation has advanced to 90° from the original position, the field will be completely dark. The Nicols are now said to be crossed, and the light which passes through the polariser is completely stopped by the analyser. A microscope provided with the two Nicol's prisms, as described, is called a polarising microscope.

If there be now placed on the stage of the microscope, the Nicols being crossed, a preparation composed of a singly refracting or isotropic substance, this will remain invisible whatever its position may be, because it transmits the light unchanged, and consequently the light which has passed through it is stopped by the analyser.

But if the preparation consists of a doubly refracting or anisotropic substance, then it will be found that this, in certain positions, appears bright on the dark field, and is consequently clearly visible. This is due to the property which anisotropic substances have of modifying a ray of polarised light transmitted through them. They decomposed the polarised ray into two, which traverse the substance with different velocities. Hence these substances are said to possess the property of double refraction. Both of the rays which leave the substance are polarised, but in planes at right angles to one another, and neither of these planes is the same as that of the original ray which is transmitted to the substance by the polariser. As it is only light polarised in this plane which is completely stopped by the analyser, a certain part of the rays which leave the object can traverse the upper Nicol and reach the eye. Hence the object appears as a luminous body on the otherwise dark field.

When a vertical section of articular cartilage is examined with the polarising microscope, the Nicols being crossed, it is found that the most superficial layer, or that next the cavity of the joint, is bright, the second dark, the third and fourth bright, being separated from one another by a narrow dark line.

In the second layer, where the cells are rounded and evenly distributed, the intercellular substance is isotropic and has no effect on the light. But where the tissue is subjected to strain, either by compression, as in the superficial stratum, or by the rapid growth of the cells in one direction, as in the deeper layers, then the intercellular substance becomes anisotropic, and appears luminous on the dark field.

This observation, which we owe to Professor Ranvier, shows, as pointed out by him, that we must not conclude at once because a tissue presents in one part the properties of single, and in another of double refraction, that it therefore consists of two altogether different substances, since the same substance may under different conditions behave differently in its action on polarized light.

5. Sections of the **Costal Cartilages of an old person** should be made with a scalpel, as the calcareous matter would

destroy a razor. The earthy granules will be seen around the cells, which have very thick capsules, frequently arranged one inside the other, so that one capsule may contain several cells each with its own capsule, and these secondary capsules may contain not one but several cells, each of which is surrounded by a tertiary capsule. In some places the intercellular substance will be seen to be composed of stiff fibres.

6. To see fat in the cartilage cells, small pieces of cartilage should be placed for twenty-four hours in one per cent. osmic acid solution, washed in distilled water, and the sections examined in glycerine. The fat drops appear black. Such preparations show, also, all the structural peculiarities of cartilage in an admirable manner.

7. To see the glycogen, sections of fresh cartilage should be stained in a dark brown-coloured solution of iodine (iodide of potassium 2, water 100, iodine to saturation). The cells which contain glycogen stain a deep brownish yellow. The intercellular substance, including the capsules, does not stain, or does so very feebly. These preparations, however, do not keep. In general glycogen is most apt to be found in young, growing cartilage; fat when the tissue is old.

8. Very instructive preparations of cartilage may be made by the following methods:—

(*a*) A very small piece of perfectly fresh cartilage is placed for an hour in chloride of gold solution, 0.5 per cent.; washed in distilled water, and exposed to the light in water slightly acidulated with acetic acid, until it becomes of a deep violet or black colour. Sections are made and examined in glycerine. The cells, which still completely fill their capsules, are dark; the intercellular substance colourless.

(*b*) Thin sections of perfectly fresh cartilage are rapidly made with a dry razor, and at once placed in one-half per cent. solution of nitrate of silver, where they are left for five minutes; then washed in distilled water, mounted in glycerine, and exposed to the light until they assume a brown colour. It is then seen that the intercellular substance is stained brown, and in this are clear spaces, which are the cavities in which the cells lie. The cells themselves are perfectly colourless and invisible. Here, as in other cases, the silver and gold methods are complementary one of the other. Silver stains the intercellular substance (chiefly the cementing material) and spares the cells; while gold fixes itself in the protoplasmic structures, and is not reduced in the intercellular substance. The appearances in the one case may be called negative, in the other positive, as regards the cells.

9. Sections of fresh cartilage placed in a closed vessel in lime water for some days, washed in distilled water, and examined in water, sometimes show fibrillation of the intercellular substance, bands of fibres extending from one cell to another. Salt solu-

tion, ten per cent., will give similar appearances. These methods often fail.

10. A piece of the **Cartilage of the external Ear** of a cow, either fresh or after picric acid and alcohol, is fixed in a piece of cork and cut. The sections should, if possible, go through the whole thickness of the cartilage, and must be very thin, as reticular cartilage is a very opaque tissue. They may be examined if fresh in salt solution, or in glycerine if hardened. The cells, enclosed in their capsules, are seen lying in a hyaline structureless basis substance. This is traversed by a network of branching and anastomosing elastic fibres, which are coarse in the central parts, but thin out towards the surfaces. They form beautiful basket-work arrangements about the cells. Near the perichondrium the cells are flattened. Acetic acid has no effect on the fibres. In sections stained with picrocarmine the cells are red, the fibres yellow, the hyaline substance unstained. The most beautiful appearances are got by double staining, first in logwood, then in eosin. The cells and fibres are red, the hyaline basis substance blue.

11. **Human Epiglottis,** preferably of a child. The network is much closer and finer, and the hyaline material in less quantity than in the ear-cartilage. Sections as before; examine in dilute glycerine.

12. Sections through the **Arytenoid Cartilage,** including the vocal process and part of the base. In the latter the tissue is hyaline; in the former, reticular. Between the two the gradual appearance of elastic threads in the hyaline material and of pericellular granules may be noticed. Double staining with logwood, and eosin gives good results.

13. **Fibrous Cartilage** will be found in longitudinal or transverse sections of the mouse's tail stained in gold chloride, and which has been already described.

A portion of the vertebral column of a rabbit or cat, decalcified by the methods to be described in speaking of bone, is cut longitudinally through an intervertebral disc and the adjacent vertebral bodies. Sections are stained in picrocarmine, and mounted in glycerine. Such preparations are very instructive, showing bone, hyaline cartilage, fibrous cartilage, and ordinary fibrous tissue. The transitions between the three latter tissues are well seen. The direction of the fibres is very oblique, almost horizontal, passing from one vertebra to the next. The bundles cross each other at right angles. Between them may be seen the cartilage cells in their capsules lying singly, or more frequently in long rows.

V. Bone:

Like the other connective tissues, bone consists of cells and of a fibrous intercellular substance, but it differs from all the tissues hitherto described in having its intercellular substance impregnated with calcareous matter, and to this it owes its density and firmness. **The fibres** of the basis substance of bone are exceedingly fine, and resemble closely those of white fibrous tissue. They are, for the most part, arranged in bundles, and are held together by a **cementing substance,** with which the calcareous salts are combined, the fibres themselves being uncalcified. In the firm material, constituted by the fibres and their cement, are embedded the cells. Each **cell** lies, as do the cartilage cells, in a space in the basis substance, but, unlike the cartilage cells, the cells of bone do not completely fill the cavities in which they lie. These cavities are of a flattened oval shape, and from their surfaces and edges numerous minute canals run off, which communicate with the canals from neighbouring cavities, and in this way the entire substance of the bone is traversed by a very close network of channels, with dilatations, in which the cells lie, occurring at intervals. The dilatations are called **lacunæ;** the channels or canals are called **canaliculi.**

Osseous tissue, thus constituted, occurs in two forms, either as minute spicules and plates, which are arranged so as to form a network or spongy mass, or as a dense substance, in which with the naked eye no cavities can be detected. The former is called **spongy bone,** the latter **compact bone.** Compact bone is to be found in the shafts of the long bones and the outer layers of the flat bones, while examples of spongy bone are to be found in the ribs, sternum, bodies of vertebræ, and articular ends of long bones. Of course, as can readily be seen in longitudinal sections of a long bone, the transition from spongy to compact bone is not abrupt, but gradual, the plates and spicules of the former becoming progressively thicker and the intervening spaces smaller. When osseous tissue occurs in small masses, as in the finer spicules of spongy bone, it contains no blood-vessels.

In compact bone, on the other hand, an abundant vascular network exists.

The basis substance of bone very commonly presents a **lamellated appearance.** This is due to a peculiar arrangement of the fibres and their bundles. In some cases these are felted together in an irregular manner, as the white fibrous bundles are in the skin and subcutaneous tissue. Here there is no appearance of lamellæ. In other cases the bundles all run parallel one to the other, as the white fibrous bundles do in tendon. Here also the lamellar structure is absent or indistinct, being due when present to the separation of layers of bundles of fibres by thin layers of cementing substance. Thirdly, the bundles may be arranged in layers, all the bundles of one layer being parallel, but crossing those of the next at an angle which may be more or less open, resembling the structure of certain membranes. Here the lamellated arrangement is distinct. The maximum of distinctness is reached when the layers cross each other at right angles.

The lacunæ lie sometimes in the lamellæ, sometimes in the cementing substance between. In the former case the surface of the lacuna is parallel to that of the lamella, and the long axis of the lacuna lies in the direction in which the fibres of the lamella run. Consequently, if the lamella be cut at right angles to its fibres, the lacuna will appear as a short, narrow, oval figure; if in the length of the fibres, but at right angles to the plane of the lamella, the lacuna will appear as a long, narrow, oval figure; while if the section be made in the plane of the lamella, the lacuna will appear as a broad oval, or, sometimes almost round figure. In each case, of course, the canaliculi passing off from its periphery will be seen.

Finally, the lacunæ and canaliculi are surrounded by a layer of substance which differs from the rest of the basis substance, and is somewhat analogous to the capsules of the cartilage cells. This substance resists the action of acids much more than the rest of the basis substance does, so that continuous portions of it can be isolated, which have the appearance of branching and anastomosing cells. That this appearance is not due to the bone cells themselves is shown by the fact that it can be got as well from macerated as from recent bone, although, of course, in the former case, the cells, as well as the other soft structures of the bone, have been destroyed (Neumann). From the recent observations of Broesike it would appear that this substance, which forms a wall for the lacunæ and canaliculi as well as for the Haversian canals, is composed of keratine. The flat bone cells, although they present projections corresponding to the orifices of the canaliculi, do not, except in very young animals, send processes into these canals, which are occupied solely by an albuminous fluid, lymph. The bone cells consequently do not anastomose. In the bones of adults the bone cells commonly atrophy and disappear.

The shaft of a long bone is a hollow cylinder of compact substance. Near its middle there is little or no cancellous tissue, but towards the extremities the compact tissue becomes gradually rarefied and increased in thickness, and passes into the cancellous mass of which the articular ends consist. The compact shaft is supplied freely with blood-vessels, which run in canals hollowed out in the bone. Of these vessels there is one, the so-called nutrient artery, which traverses obliquely the thickness of the shaft of the bone, and is distributed chiefly to the medullary tissue and walls of the medullary cavity. Innumerable small vessels penetrate the surface of the bone by pores obliquely situated, and which can be seen with a lens as fine openings all over the surface of the dry bone. From the medullary cavity other small vessels penetrate the bone from within in a similar manner. All these vessels combine to form a close network with elongated meshes, the long axis of the meshes being in the long axis of the bone. The channels in which this network lies are called **Haversian canals,** and from their arrangement it is clear that on a transverse section of the bone most of these canals will be seen cut across, while on a long section they will be for the most part divided longitudinally. But on sections made in either direction the retiform arrangement of the Haversian canals will be evident, as well as the channels by which the network opens on the outer and inner surfaces of the shaft of the bone.

The tissue of the compact bone is markedly lamellar, the fibres of one lamella forming a right angle with those of the next; and as these fibres run, for the most part, either in the length of the bone, or at right angles to this, sections in either of these directions will divide some of the lamellæ parallel, others at right angles, to the course of their fibres. The former lamellæ will appear striated; the latter punctated.

The **arrangement of the lamellæ** is as follows:—

On the surface are a number of lamellæ, which encircle the bone. These are called the **circumferential, or outer fundamental lamellæ.** Surrounding the medullary cavity are other lamellæ, which are called the **inner fundamental lamellæ**. Between these two sets the arrangement of the lamellæ is more complicated. There are—first, lamellæ which surround each Haversian canal. These are called **Haversian lamellæ**, and, together with the canal forming their centre, constitute what is called a **Haversian system**. Each Haversian system is marked off from the rest of the bone by a fine line formed of cementing substance. Secondly, between the Haversian systems are found irregularly-shaped patches, which contain no vessels, and in which the lamellæ are arranged parallel to one another, and also to the fundamental lamellæ. These are the **interstitial lamellæ**, and might be called the middle fundamental lamellæ. Thirdly, there are larger or smaller fragments, in which the arrangement of the lamellæ is such as to show that they are parts of a Haversian system, the remainder of which has been absorbed, and its place taken by a new system, the centre of curvature of whose lamellæ is, of course, different. These three arrangements of lamellæ can be recognized either on transverse or on longitudinal section of the bone, but are most distinct in the former.

In the long bones of some small animals, where the quantity of osseous tissue is scanty, the entire bone forms but one Haversian system; all the parts are arranged concentrically around the medullary cavity, and there are no blood-vessels in the bone itself. In the compact tissue of larger bones the lacunæ of all the fundamental lamellæ form one system, communicating by their canaliculi, and the canaliculi of the innermost lacunæ open into the medullary cavity. The innermost lacunæ of each Haversian system open by canaliculi into the Haversian canal, and a free communication exists between the different lacunæ of each system by means of their canaliculi. But the canaliculi of the outermost set of lacunæ of each system communicate only to a very slight extent with those of the surrounding portions of bone. They generally turn back into their own system, and reopen into some of its lacunæ. They are called recurrent canaliculi (Ranvier. Von Ebner).

Besides the parts already mentioned, bands of fibres pass from the surface of the bone, and penetrate at various angles through the lamellæ of the fundamental systems. These fibres often anastomose, and form a network. They never enter the Haversian systems. They are better developed in the flat bones of the cranium than in the long bones. They consist of fibres resembling white fibrous tissue, with a calcified cementing substance. They are called **Sharpey's perforating fibres**.

There is also to be found in the long bones a certain quantity of **elastic tissue**, formed of branching and anastomosing fibres, which differ in their chemical character from the other fibres of the bone.

The compact bone passes into spongy tissue by a gradual enlargement of the Haversian canals, which at last open out into the areolæ of the cancellous tissue. The portions of bone bounding these areolæ vary greatly in thickness. In all, the lamellar structure exists, and the larger contain Haversian systems, while the thinner spicules and plates have no proper vessels, but are nourished only by means of the lacunæ and canaliculi.

In the medullary cavity of the long bones of healthy adults is contained a tissue which differs from adipose tissue only in the fact that the fat cells are not separated into groups or lobules by fibrous tissue, and that the distribution of blood-vessels is not so regular and abundant. This is **yellow marrow.**

In the areolæ of the cancellous tissue of the short and flat bones, and of the epiphysary ends of the long bones is contained a peculiar tissue, the **red marrow.** This consists of a network formed of branching and anastomosing cells, in the meshes of which are contained. 1. Numerous cells, resembling those of lymph, and which differ a good deal in size and in the abundance of contained granules. 2. Larger cells, whose nuclei are frequently of very irregular shape, and furnished with a number of buds, or projections. 3. Very large cells, containing a considerable number of nuclei (giant cells). 4. Blood corpuscles, many of which are often of irregular shapes and variable sizes. 5. Nucleated cells, whose substance is smooth and homogeneous, and impregnated with hæmoglobin (nucleated red blood-corpuscles). 6. Fat cells. It is in the cells of the second and third variety that the process of nuclear division has been observed, which has been named fragmentation, and which has been already alluded to.

The blood-vessels of this tissue are numerous, particularly the veins, whose walls are extremely thin, and, in places, composed only of the medullary tissue itself, so that solid bodies can freely pass from this into the blood-current (Rindfleisch). If the tissue is not fresh there are frequently found in red marrow long octahedral crystals, They are supposed to be formed of the phosphatic salt of an organic base, which, from its occurrence in the secretion of the prostate, and consequently in the semen, is called spermatin. They are commonly known as **Charcot's crystals**.

Besides the red and yellow marrow, a third variety has been described as **gelatinous marrow**. It is a modification of the red, and occurs generally in atrophic or cachectic conditions. The fat cells are absent, there are much fewer red corpuscles, and the whole tissue is impregnated with a mucinous fluid.

It has been stated recently, that in the bones of the extremities red marrow is either altogether absent, or confined to the upper ends of the humerus and femur, the marrow in the lower ends of these bones and in those more peripherally situated being exclusively yellow (Neumann).

A small quantity of medullary tissue surrounds the blood-vessels in the Haversian canals.

The bone is surrounded by a membrane of considerable strength and density, the **periosteum**. This is more closely adherent at the extremities of the long bones than in the middle part, and is peculiarly difficult to separate at the insertion of tendons. It consists of two layers—an outer layer, which is composed of fibrous and elastic tissue, and an inner layer, which is more cellular. The inner layer is very highly developed in growing bones, and plays a most important part in the formation of the new osseous tissue. Sharpey's fibres are continuous with the periosteum. The blood-vessels in the periosteum are numerous, and from them the vessels pass into the interior of the bone.

Bone has to be examined by **two methods.** The first method consists in removing the earthy salts from the recent bone by the prolonged action of a dilute acid, and then in making sections with a razor of the softened decalcified tissue. Preparations made in this way show not only the structure of the basis substance, but also the soft parts of the tissue, such as the bone cells, the periosteum, the blood-vessels, and the medulla. By the other method the bone is submitted to prolonged maceration in water, by which all the soft structures are destroyed and removed, while the calcified basis substance remains. This is ground down into thin plates, which are examined in Canada balsam. Many details in the structure of the bone, particularly the arrangement of the lamellæ, and the lacunæ and canaliculi, are best seen in preparations made by this method.

First Method:

When Bone is to be softened in acid it must be divided into small pieces, and placed in a large quantity of the acid fluid, which should be changed frequently until the decalcification is completed. The best acid to use is a mixture of chromic and nitric acids, half of the former, and one of the latter to 100 of water. The chromic acid prevents the swelling of the fibres of the basis substance, which would otherwise be caused by the mineral acid. The bone should be placed fresh in this mixture, and examined from time to time. When it is found that the pieces have become flexible, and when a fine needle passed through them in different directions meets with no gritty resistance, the tissue is decalcified. The pieces of bone are then washed in water for a considerable time, to remove the acid, and preserved in alcohol until they are wanted for section.

The sections are made with a razor, as described for cartilage, the piece of bone being fixed into a split in a cork. The sections may be mounted in dilute glycerine, either without staining, or after being stained in picrocarmine, logwood, or purpurine. The preparations may be made permanent by cementing the cover-glass in the usual way.

Compact Bone.—1. Transverse section through the Shaft of a Long Bone. This should include the entire thickness of a segment of the ring of compact bones of which the shaft consists. It is best, therefore, to use the bone of some small animal, as a cat or dog.

Make first a general examination with a low power, and then study the details with a higher power.

Most of the Haversian canals are cut across, and appear as round figures. Since, however, they really form a network, some canals are cut in length, or obliquely, and canals opening on the inner or outer surface of the bone may possibly be seen.

Bounding the outer and inner surfaces of the bone, portions of tissue may be seen which contain no Haversian canals (except such as may pass through them to open on the surface). These are the outer and inner fundamental lamellæ. In the middle portion of the bone which lies between these, the canals are distributed with more or less regularity.

Covering all parts of the section will be seen minute angular-shaped dots. These are the lacunæ. In the fundamental lamellæ these are disposed in rows parallel to the surfaces of the bones, while surrounding each Haversian canal the lacunæ are arranged concentrically. The portion of bone about each canal, and which contains these concentrically-placed lacunæ, is marked off by a fine line, and is the cross-section of a Haversian system. On the outer surface will be seen the fibrous periosteum.

With a high power the Haversian canals will be seen to contain each one or more blood-vessels, surrounded by some round medullary cells. The shape of the lacunæ will be distinctly seen, and it will be noticed that while they all are narrow elongated figures, some are much longer than the others. The longer ones are those which are placed in the lamellæ whose fibres lie in the plane of the section, the shorter those contained in lamellæ whose fibres are cut across. Each lacuna contains the round nucleus of the bone cell, and if the preparation be good, there may be seen running off from opposite poles of the nucleus a thin streak of protoplasm, the profile view of the bone cell itself. In old bones the cells may have altogether disappeared, while in young bones they are very conspicuous objects.

The canaliculi, if visible at all, will be very faint and indistinct, owing to their being filled with fluid of nearly the same refractive power as the bone.

The lamellæ will be more or less distinctly seen. They and the canaliculi will be more apparent in preparations made by another method.

In the periosteum it will be observed that the greater number of the bundles of white fibrous, and threads of elastic tissue are cut transversely.

2. **Longitudinal Section of the Shaft of a Long Bone.**—The Haversian canals will be seen cut in length, and anastomosing by cross-branches, and so forming a network with elongated meshes. The lacunæ are narrow, elongated figures, lying parallel to the Haversian canals. The outlines of the Haversian systems are indicated by lines parallel to and at some distance from the canals. The fibrous bundles of the periosteum are cut chiefly in length.

Spongy Bone—3. Section through the Articular End of a Long Bone.—The osseous tissue will be seen to form a network, enclosing large, irregularly-shaped spaces, which contain round cells, lying in a fine reticulum, and traversed by blood-vessels. This is red marrow. The osseous network consists of plates of bone of various thickness. In the thicker, blood-vessels, lying in Haversian canals, surrounded by their systems of lamellæ and lacunæ, are present, but in the smaller, lacunæ only are visible. These are generally placed with their flat sides parallel to the surface of the osseous tissue.

4. From a piece of compact decalcified bone, remove the periosteum, and with a scalpel scrape the surface of the bone. Examine the fragments of the superficial lamellæ, which come off in a drop of water. At the thin edges it will be seen that they consist of bundles of very fine fibres, which cross each other at very sharp angles (Sharpey. Von Ebner).

Other acids, besides the mixture of chromic and nitric acids, may be used to decalcify bone. Of these, chromic acid, half per cent., or picric acid in saturated watery solution, are most employed. Both these acids act very slowly, and require the bone to be cut into extremely small pieces. They have certain advantages, but for ordinary purposes are not so good as the chromic and nitric mixture.

Second Method:

In preparing a Bone for making grindings certain points must be attended to. The bone, immediately on removal from the body, should be cut into pieces, and the medulla, as far as possible, removed by a stream of water. The pieces of bone should then, before they have time to dry, be placed in water, which should be frequently changed. In this they may be left an indefinite time, for the intercellular substance of bone, so long as it contains its earthy salts, and is not pulverized, is almost incapable of undergoing putrefaction.

If the bone be allowed to dry before being placed in the water, fatty matters will soak into the pores of the bone, to take the place of the evaporated water, and it is nearly impossible ever to free the bone from this fat. After some months of maceration, the pieces of bone may be removed from the water, well washed, and placed in the air to dry and bleach. They should be dry, white, uniformly opaque, and quite free from fatty stains.

The bone is fixed in a vice, and thin sections are cut with a fine saw. These are ground down at first on a grind-stone, and then on a fine hone, moistened with water. The piece of bone should be held against the stone by the pulp of the finger or thumb. If it is found that some parts are becoming thinner than others, the pressure on the thin parts should be relieved, while the finger is made to press more firmly on the thicker parts. The grinding should be made on both sides of the section alternately, and should be continued until the moistened plate of bone is quite transparent. The section is then well washed and dried.

The section has next to be mounted in Canada balsam, and in such a way that the balsam shall not penetrate into the lacunæ and canaliculi. If ordinary fluid balsam were used this would occur; but if the balsam has been exposed for some time to a gentle heat it becomes, on cooling, hard and brittle, and such dried balsam will solidify before it has time to soak into the canals of the bone.

A small piece of hard balsam is placed on the slide, which is warmed over a small gas or spirit flame until the balsam melts. The warming must be cautiously effected, for if the heat be too rapidly applied the balsam will boil, and the mass of bubbles will spoil the preparation. When the balsam is melted, the thin plate of bone is taken by one corner in a forceps, and quickly plunged into the balsam, and the cover-glass, which must be in readiness, rapidly applied and pressed down. The whole slide is then cooled by being placed on a large piece of cold metal, or by having a stream of water poured over it.

This method is troublesome and laborious, but the results are very good. Longitudinal and transverse sections of compact tissue should be made by this method. These preparations are permanent.

Spongy bone, owing to its fragility, cannot be cut with a saw. The piece of bone to be cut must be soaked in thick gum until all the interstices are filled. Then it is exposed to the air until the gum becomes thick, and then placed in alcohol, which completes the hardening of the gum. In this condition, with its cancelli filled with gum, the bone will bear the saw. The sections must be rubbed down on a stone moistened with alcohol, and when sufficiently thin they are placed in warm water, which dissolves out the gum. They are then dried and mounted in balsam in the way already described (Ranvier).

5. In grindings mounted in balsam the Haversian canals appear empty, or filled with bone-dust. The lacunæ and canaliculi contain air only, and appear very distinctly as black branching figures and lines. They owe their black appearance to the total reflection of the light from their lower surface, and this total reflection is due to the great differences between the refractive power of Canada balsam and that of air. If the balsam were to soak into the spaces of the bone, this total reflection would not occur, and the lacunæ and canaliculi would be invisible. It is probable that at some part of the edge of the preparation this will have occurred. It is to prevent its occurrence that the mounting has to be carried out with the precautions above indicated. It is easy to see that the air in the lacunæ reflects the light. If, while the preparation is examined with a low power, and the lacunæ appear black, the hand be placed before the mirror of the microscope, so as to shut off the light passing through the object, the lacunæ will be seen as bright spots on a dark ground. They now reflect the light,

which falls on them from above. If light be concentrated on the preparation by a lens, the brightness is of course greater.

On cross section through the shaft of a long bone the lacunæ all appear as narrow objects, but on long section many lacunæ will be seen from their flat surfaces, and appear as broad, oval, or round figures. It will be seen that the canaliculi come off mostly from the surfaces of the lacunæ; that they are commonly branched; that those of neighbouring lacunæ anastomose; that the innermost canaliculi of a Haversian system open into the canal, while the outermost generally form loops, and return into their own systems, anastomoses with the canaliculi of neighbouring systems, or of intermediate portions of bone occurring only occasionally.

The irregularly-curved outlines of the Haversian systems are distinct, and it will be seen that the bone consists of a sort of mosaic of fragments of fundamental lamellæ and of Haversian systems, some in process of formation, others of absorption (Von Ebner). This is due to the fact that bone grows, not as other tissues do, by interstitial expansion, but by the continual absorption of old parts, and laying down of new.

The arrangement of the lamellæ is evident. On examination with a high power the lamellæ will be seen to present alternately a homogeneous, or finely striated, and a granular, or finely punctated, appearance. The former appear brighter than the latter. The former are those whose fibres run in the plane of the section; the latter those whose fibres are cut at right angles. If the Canada balsam has soaked into the canaliculi, the punctated lamellæ will present shining lines, passing through them at right angles to the plane of the lamellæ. These are the canaliculi, filled by the hard, highly-refractive balsam. It will be noticed that the lacunæ do not all lie between the lamellæ, but that some lie in their interior, and that, according as they lie in a clear or in a punctated lamella, the shape differs, as already described.

6. A balsam preparation should be examined in polarised light with the Nicols crossed, when the lamellar structure will be very beautifully seen. The punctated lamellæ are dark; the striated lamellæ bright, except along two planes, at right angles to one another, in which they also appear dark. Hence, on transverse section, each Haversian canal is surrounded by circles, alternately bright and dark, the whole being marked by a black cross, whose arms meet in the central canal. Where the intermediate lamellæ are arranged parallel to either arm of the black crosses of the Haversian systems, they are all dark; where they run obliquely to the crosses, they appear alternately bright and dark, according as their fibres are cut in length, or at right angles. These appearances can also be seen, although less brilliantly, in decalcified sections, mounted in glycerine or water.

They are due to the fact that light, when it passes through the fibres of connective tissue in a direction parallel to their length, undergoes no change; hence the punctated lamellæ are dark. But when it passes through the fibres obliquely, or at right angles to their length, it undergoes double refraction; hence the striated lamellæ are bright. If the fibres, however, lie parallel to the axis either of the polariser or of the analyser, the light is completely stopped, and in this way are produced the black arms of the rectangular crosses.

7. **Sharpey's fibres** occur in the fundamental lamellæ of long bones, but are more developed in the flat bones forming the roof of the skull. A portion of one of these is decalcified, and sections are made perpendicularly to the surface. Shining bundles of fibres will be seen passing perpendicularly or obliquely through the superficial lamellæ, and frequently forming a network in the interior of the bone. They never penetrate the lamellæ of a Haversian system. They are continuous with the fibrous tissue of the periosteum, and occur only in bone which was developed from this membrane.

When in such a section the lamellæ are torn asunder with needles, it can be seen how they are perforated by Sharpey's fibres.

8. From a broken rib, or from the cancelli of the body of a vertebra, some of the red pulpy **medulla** is taken with the point of a knife, and gently torn up with needles in a drop of half per cent. salt solution. The medulla from the cavity of a long bone of a young rabbit or guinea-pig is a good object for examination. The bone should be broken, or split in length with a strong knife, to avoid the mixture of bone dust which would occur if the bone were sawed (Ranvier). The different kinds of cells described above should be looked for.

9. A fragment of the medulla is torn up in salt solution on a slide. This is inverted, and placed for a few minutes over the open mouth of a bottle, containing some one per cent. solution of osmic acid. The vapour of the acid fixes and hardens the cells. A drop of picrocarmine is then placed on the preparation, and this is left in the moist chamber for some hours. A cover-glass is then applied, and a drop of glycerine placed at the side, and allowed to diffuse in and take the place of the picrocarmine. The excess of glycerine is then removed with filter-paper, the slide cleaned, and the preparation sealed with cement.

CHAPTER VI.

MUSCULAR TISSUE.

MUSCULAR tissue occurs in three forms—1. As unstriped muscle. 2. As the tissue of the myocardium. 3. As the striated skeletal muscles.

I. **Unstriped muscle** forms the contractile coat of the stomach, intestines, bladder, uterus and Fallopian tubes, and blood-vessels. It occurs also in considerable quantity in the skin, in the prostate, in the interior of the eye, and in other parts.

It consists of cells, which are usually spindle-shaped, but sometimes branched. In the middle of each cell is a long rod-shaped nucleus, in which a very distinct intranuclear network can often be seen. The substance of the cell is obscurely striated longitudinally, and would seem to consist of contractile threads set in a homogeneous protoplasm, which is collected in somewhat greater quantity about the nucleus. The outlines of the cell are generally smooth, but are sometimes marked by ridges or irregularities, due to compression of neighbouring cells. There is no demonstrable cell-wall. The appearance of transverse striation, which is sometimes seen on these cells, is probably due to markings on the surface only. In warm-blooded animals the cells are from $\frac{1}{1200}$ to $\frac{1}{500}$ inch long.

A cementing substance connects these cells together into bundles, which either run singly, or more commonly are united by connective tissue, so as to form membranous structures such as the walls of the hollow viscera. In the junction of the individual cells, the sharp end of one cell is wedged in between the thicker parts of two others, so that on cross section through a bundle the cells appear as circular or polygonal figures of very various sizes, since some are cut near their centres, others near their ends. In the former case a nucleus is seen, in the latter not.

When a portion of smooth muscular tissue is examined fresh, its composition of spindle-shaped cells is not always evident, but it appears to consist of a fibrous substance, in which long nuclei are set at regular intervals. The cellular structure of the tissue has been known only since the methods of isolation have been introduced into histology, for the cells cannot be separated until their cementing substance has been softened.

Smooth muscular tissue is freely supplied with blood-vessels, which form a network of capillaries, with meshes elongated in the direction of the bundles of cells. Associated with smooth muscular tissue numerous ganglia are commonly found. These and the distribution of nerves will be subsequently described.

II. **Muscular Tissue of the Heart.**—Here, as in the unstriped muscle, the tissue is composed of cells of microscopic size. In the lower vertebrates the cells are of spindle shape, but in the higher classes of animals they are short cylinders. In neither case is the outline of the cells smooth and even, but presents numerous crests, ridges, and projections, which are places where one cell meets with and is cemented to another. Each cell shows a distinct transverse striation, which does not differ from that of the voluntary striated muscles. A longitudinal striation also exists, and is due to the presence of groups of fibrils, which lie side by side, and are embedded in a homogeneous protoplasm. The transverse striation is due altogether to the structure of the fibrils, which consist of alternate dark and light portions. In the centre of each cell is an oval nucleus surrounded by a certain quantity of protoplasm. Two nuclei are sometimes present. There is no cell-wall or sarcolemma.

These cells are cemented one to another, so as to form a network with long and narrow meshes. This network is not extended in a single plane, but in all directions, so as to form a spongy tissue. Portions of this tissue in the shape of bundles and laminæ are bounded by layers of connective tissue, in which the larger blood-vessels lie. Since, without the use of reagents, the cellular structure of the myocardium is usually not apparent, the tissue seems to consist of branching and anastomosing fibres, containing oval nuclei at intervals.

The blood-vessels are numerous, and form elongated meshes. They commonly lie in spaces between the muscular bundles. These spaces are the commencements of the lymphatics. In the heart of the frog there are neither blood-vessels nor lymphatics.

III. **Striated Skeletal Muscles.**—This tissue is composed of **cylindrical fibres,** with pointed ends. They can be seen with the naked eye, and have frequently a length exceeding an inch. The breadth varies considerably in different fibres. These fibres lie side by side, and are held together in coarse bundles by connective tissue, which sends septa into the bundles, dividing them into smaller groups, and these again into still smaller. The connective tissue surrounding the bundles is called **perimysium**, that between the fibres **endomysium**. The smallest bundles are named primary, the larger secondary and tertiary.

The muscular fibres do not anastomose with one another, and very rarely branch.

Each fibre is enclosed in a structureless sheath, which completely surrounds it. The sheath is called the **sarcolemma.** Within it is contained the **contractile substance.** This presents a distinct transverse striation, the exact pattern of which varies greatly in the muscles of different animals and in the same muscle, according as it is stretched or relaxed, contracted or not. The **details of the trans-**

verse striation can be seen better in the muscles of insects and crustacea than in those of mammals, as in the former animals the striæ are much broader and the markings coarser. In a fibre which is not contracted, and which is moderately stretched, the following markings may be seen. At regular intervals the fibre is crossed by—(1) a narrow, highly-refracting line, which appears dark and frequently granular. This has been called *Krause's membrane*, the *thin disc*, and many other names. At this line the sarcolemma is more adherent to the contractile substance than at other points. Passing along the fibre from a thin disc, we next meet (2) a clear stripe. This is followed by (3) a broad, dark band (*broad disc*), but which is less highly refracting than Krause's membrane, and which in insects' muscles can be clearly seen to be composed of a number of rod-like bodies lying side by side. This is followed by (4) another clear, feebly refracting stripe like (2); and finally we come on another thin disc or Krause's membrane. This much can readily be seen on the muscles of mammals. In insects' muscles there are sometimes other bands seen, two narrow granular bands lying one on each side of and close to Krause's membrane (*accessory discs*), and a band occupying the middle of the broad disc (*intermediate disc*, or *Hensen's disc*). When muscle is examined in polarised light, it is found that Krause's membrane (1) and the broad disc (3) are doubly refracting, while the two clear discs are singly refracting. In consequence of this, a muscular fibre suitably placed in the dark field of the polarizing microscope appears composed of alternate dark and light transverse bands. The accessory discs are doubly refracting in a feeble degree. Hensen's disc would appear to be singly refracting.

When a muscular fibre is subjected to the action of certain reagents, as alcohol, chromic acid, bichromate of potash, &c., it can readily be divided into a number of fine threads, each of which shows the same pattern of transverse striation as the entire fibre. These threads are called **fibrils;** and since the fibre consists of a number of these fibrils, it is sometimes called a **primitive muscular bundle.** On cross section of a muscular fibre, the cut surface presents a series of highly refractive points, separated by a network of lines of a less highly refractive material. These points are called **Colmheim's fields,** and they correspond, not to the cut ends of individual fibrils, but to groups of these which are called **primitive muscular cylinders,** and are separated by tracts of protoplasm. This protoplasm is much more abundant in the muscle of the heart than in the voluntary muscles. The primitive cylinders in the myocardium are consequently very distinct.*

When a muscular fibre is submitted to the action of dilute mineral acids, or when it is frozen and thawed again, it cleaves, not into fibrils, but transversely into **discs,** the cleavage always occurring through a clear band close to Krause's membrane. In consequence of the double cleavage into fibrils and into discs, it has been supposed that the contractile substance really consists of little prismatic-shaped particles **(sarcous elements)** cemented together, and that the cementing substance which joins them end to end is different from that which joins them side to side. One class of reagents dissolves the latter cement, and causes the division into fibrils; another dissolves the cement joining the particles end to end, and causes the transverse cleavage of the fibres. This will be clear if we suppose a number of rods of wood to be glued to one another to form a bundle, and if we suppose, when the glue is hard, this bundle cut transversely into a number of discs, each of which will contain a portion of each rod. If we further suppose these discs then cemented together in their original positions, not by glue, but by a resinous cement. If then the whole bundle be placed in water, the glue will dissolve, and the rods will separate one from the other, the resinous cement not being affected, and each rod will consist of alternate layers of wood and of resin—will show, in fact, a transverse striation. If, however, the bundles are placed in alcohol, this will not affect the glue, but will dissolve the resin, and the bundle will break across into discs, and each disc will show a number of particles of wood separated by a network of glue.

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* Recent observations tend to show that the network seen on cross sections is not due to membranous septa bounding cylindrical groups of fibrils, but to a network of threads pervading the fibre (Retzius. Bremer).

The intimate structure of the contractile substance of muscle is, however, a subject on which so much uncertainty still prevails, that it would be useless to say more about it here.

Besides the contractile substance, there is contained within the sarcolemma a number of **nuclei.** These are oval in shape, with their long axes parallel to the fibre, and have about them, particularly at their ends, a certain quantity of protoplasm. In the muscles of the frog the nuclei lie at all depths in the fibre, but in mammals most of the muscles have the nuclei situated immediately under the sarcolemma. In each fibre a very large number of nuclei are contained.

It was formerly supposed that, at the **attachment of a muscle to its tendon,** a direct continuity existed between the contractile tissue of the muscles and the fibrous tissue of the tendon. This is now known not to be the case. The muscular fibre ends with a rounded or pointed extremity, and this extremity, like the remainder of the fibre, is covered by the sarcolemma. The contractile substance is, under ordinary circumstances, so closely attached to the interior of the sarcolemma, that any separation is impossible: but by certain means this attachment can be loosened, and the fibre separates readily from its sheath. It can then be seen that the tendon fibres are attached to the outside of the sarcolemma, the extremity of which is embraced by them, but that they have no continuity with the contractile substance. Whether the tendon is continuous with the sarcolemma, or only attached to this by cementing substance, is uncertain.

Of all the fibres which exist in a muscle, comparatively few join the terminal tendons, since, except in the case of the shortest muscles, the length of the fibres is less than that of the muscle itself. The fibres which do not reach the end are attached to one another by the perimysium, which serves in this way as a sort of internal tendon.

Striated muscles are abundantly supplied with **blood-vessels.** The arteries run transversely to the direction of the fibres, and break up into capillaries, which form elongated rectangular meshes, the longer diameters of which are in the direction of the fibres. Hence, on long sections most of the vessels will be cut in length, while on a section at right angles to the fibres most of the vessels will be cut across, just as was the case with the Haversian canals in the shaft of a long bone. The veins generally leave the muscles in company with the arteries.

Many animals possess muscles which differ from one another in colour, some being red, others white. The modes of contraction of these two kinds of muscle are very different. That of the red muscles is slow and sustained, while that of the white muscles is abrupt and sudden, and immediately followed by relaxation, unless the stimulus be repeated. Corresponding to these physiological differences, each kind of muscle possesses certain anatomical peculiarities. In the fibres of the red muscles the nuclei are very numerous, and do not all lie close under the sarcolemma, but also in the interior of the contractile substance. The transverse striation is less well marked on the red muscles than on the pale; and in the red muscles the capillary blood-vessels are more numerous, have a tortuous course, and on the cross branches of the network and on the commencements of the veins there are commonly little dilatations like small aneurisms (Ranvier).

The **lymphatics** form spaces surrounding the muscular fibres. These communicate with the networks of lymphatic vessels which are contained in the connective tissue sheath of the muscle.

The nerves will be described in the next chapter.

Unstriped Muscle:

1. A small fragment of the muscular coat of the intestine or stomach of a frog or mammal is placed in a forty per cent. solution of caustic potash, This solution must be put into a watch glass, which should be placed on a plate or saucer, to avoid the risk of the spilling of the corrosive alkaline fluid on the table. The watch glass must be kept carefully covered with a glass plate, to prevent absorption of water and carbonic acid from the air.

In fifteen to twenty minutes the piece of muscle will be found quite friable. A minute portion is placed on a slide *in a drop of the potash solution* and torn up with needles, covered, and examined with the high power, all fluid about the edge of the preparation having previously been removed with filter paper.

The long, spindle-shaped, smooth muscular fibre-cells will be seen, many of them single, others lying together with their attachments more or less loosened, so that their relation is easily made out.

This preparation cannot be kept, and if some water be added to it, the cells will all dissolve. It is only when the alkaline solution is very strong that it acts as a preservative of the anatomical elements; if it be more dilute, these elements are dissolved.

2. A small piece of the muscular coat of the stomach or intestine is macerated for twenty-four hours in twenty per cent. nitric acid, and then washed in water. Fragments are then torn up with needles, and examined in water or dilute glycerine. The cells are seen as in 1, but they are frequently corrugated and wrinkled. Their nuclei are fairly distinct.

3. The bladder of a frog is removed and laid open with scissors, and then placed for twenty-four hours in diluted alcohol (rectified spirit 1, water 2). It is then placed in water, and with a camel's hair brush the epithelium of its inner surface is carefully brushed away. Thin portions are then cut off, stained in logwood or picrocarmine, washed in water, spread carefully on the slide, and covered in a drop of glycerine. With a low power, or even with the naked eye, a coarse network will be seen, and in the spaces enclosed by this the microscope will show a finer network. Both of these networks are formed of smooth mus-

cular tissue, which in the bladder of the frog does not form a continuous membrane, as in the bladders of higher animals, but merely a reticulum, which is set in a continuous layer of connective tissue. In the larger bands the outlines of the individual cells may not be visible, but the elongated nuclei will be well seen. In some of the larger bands arteries will probably be found. They run a tortuous course, and the transversely-placed muscle cells of their middle coat are evident. The smaller bands consist each of only a few muscle cells, whose shape and arrangement will be easily made out. Many completely isolated cells will be found in the interspaces of the network. Some of these are not spindle-shaped, but stellate, with three or more rays. Between the muscle cells will be seen the fibres and the round or oval nuclei of the cells of the connective tissue.

4. A much better method of preparing the bladder is as follows :—The cloaca of the frog is ligatured close to the anus. This is best done from behind. The urostyle is carefully removed, when the ligature can be readily applied. The animal is then laid on its back, and the abdomen opened down to the pubis. The large intestine is opened, and its contents pressed out. A glass cannula is tied into the opening, and dilute alcohol is forced into the intestine by a syringe, or allowed to flow in from a vessel elevated eighteen inches or so above the animal. The alcohol fills the large intestine, and rapidly passes from it into the bladder, which it distends fully. When this is accomplished the bladder is ligatured at its base, and cut away below the ligature, and placed in a vessel containing dilute alcohol. In half an hour it may be opened, when it will be found that the muscular fibres show no tendency to retract, but the viscus remains in the fully distended condition. The bladder is left in the dilute alcohol for twenty-four hours. The epithelium is then macerated, and may be readily removed by brushing. Portions of the fibro-muscular coat are stained first in logwood, then in eosin, washed, floated on to the slide, carefully spread, and mounted in glycerine. The cells are red, the nuclei blue, the fibrous connective tissue scarcely stained. The outlines of the cells, even where they lie in thick bundles, are distinct, and the appearances are in every way superior to those seen in preparations in which the bladder was not distended prior to the death of the muscular tissue.

These preparations may be cemented and kept permanently.

5. In the mesentery of the newt there is a plexus of smooth muscular tissue, in which the cells are of very large size. In their oval nuclei a very beautiful reticulum may be readily demonstrated. The mesentery, with the intestine attached, is placed for twenty-four hours in five per cent. neutral chromate of ammonia. Then the intestine is carefully separated, the mesentery washed in water, stained in logwood, and mounted permanently in glycerine (Klein).

In the examination of the digestive tube and other organs frequent opportunities will be found for the study of sections of smooth muscular tissue.

Myocardium :

6. Portion of the wall of the ventricle of the heart of a recently-killed animal (rabbit or guinea-pig) is snipped off with scissors in the direction of the fibres, and slightly torn up with needles, and examined in a drop of salt solution. It will be seen that the fibres are transversely striated, and that they form a network, with long, narrow meshes.

Another piece may be stained in picrocarmine, and mounted permanently in glycerine. The nuclei will be red, and the muscular tissue itself orange. It is probable that in neither of these preparations will anything be seen of the division into cells.

7. A fragment of the heart of a mammal, and one of the heart of a frog are macerated for fifteen to twenty minutes in caustic potash, as described above (1), and examined in the alkaline fluid. The cells will be isolated. They will be seen to be transversely striated, and their nuclei will be distinct. They are irregularly spindle-shaped in the frog, but of a short cylindrical shape in the mammal. In both cases projections from the sides of the cells will be noticed, and where the cells have not completely separated from one another, they will be seen to be joined at these projections. In the mammal the cells forming the auricular wall will be found longer and narrower than those in the ventricle.

8. A portion of the ventricle of a recently-killed animal, still covered by its endocardium, is washed in distilled water, and placed in half per cent. nitrate of silver for an hour. Then a part of the endocardium, about a quarter of an inch square, is circumscribed by four shallow incisions, and torn off with forceps, carrying with it a thin layer of muscular fibres. These are mounted in glycerine, with their deep surfaces uppermost, and exposed to the light. The division between the muscle cells will be marked by black lines (Ranvier).

9. The ventricle of a rabbit, cat, &c., is hardened in quarter per cent. chromic acid. Sections are stained in logwood, mounted in glycerine or balsam. The transverse sections of the muscular fibres appear as very irregularly-shaped figures, in consequence of their frequent branching and anastomoses. The nuclei will be seen situated, not at the surface, but in the interior of the fibres, and the dotted appearance of the latter is distinct, owing to the large quantity of protoplasm which lies between the primitive cylinders.

Skeletal Muscles :

10. The contractile substance of muscle undergoes a coagulation after death, by which its consistence is increased. It is

best to examine the fibres after they have undergone this *post-mortem* coagulation, or *rigor mortis*. An incision is made into the rigid muscle parallel to the direction of the fibres, and from the raw surface a very small fragment is detached with scissors or forceps, and placed on a clean slide, and dissociated with needles. It is best to add no fluid, as the fibres will thus adhere to the glass wherever they are placed, and not float away from the needles; but the preparation must at no time become completely dry, and to avoid this it should be frequently breathed on while the isolation of the fibres is being carried out. This should be accomplished with as few touches of the needle as possible, for wherever the needle touches the fibre there its structure is injured or destroyed. The progress of the dissociation should be examined from time to time with a low power, without applying a cover-glass. When a few fibres are completely separated from the others, a drop of salt solution should be placed on the cover-glass, which is inverted, and gently placed on the object. The fibres will appear with smooth outlines. The transverse striation is distinct, consisting of alternate dim and clear stripes. If the fibres be fully stretched, the thin dark band, or Krause's membrane, may be seen in the middle of the clear stripe. By altering the focus all that was previously clear becomes dim, and *vice versa*.

In places the transverse striation is not seen, but the fibre presents a granular, glassy, or broken appearance. These are points to which the needle has been applied, and the fewer of them there are the better is the preparation. In some of these injured places the contractile substance may be altogether broken across, while the sarcolemma, which is tougher and more resistant, is seen as two fine lines extending between the broken segments. In the empty sarcolemma-tube granular fragments of the contractile substance, or free muscle nuclei, may be seen. Where the fibre is intact the sarcolemma lies so close to its contents as to be invisible.

The nuclei are not seen in the fresh muscle, because they have the same refractive index as the contractile substance in which they lie.

11. Irrigate the last preparation with dilute acetic acid. The contractile substance becomes paler, the striation less distinct. The nuclei are now clearly visible as spindle-shaped figures, placed with their long axes parallel to that of the fibre. A small quantity of granular protoplasm may be seen surrounding them, most abundant at their ends, from each of which it runs off as a fine line. This is better seen in the frog's muscles than in those of mammals. At the divided ends of the fibres the swollen contractile substance may protrude from the sarcolemma as a fungus-like mass. At the constricted base of this the sarcolemma will be seen thrown into transverse wrinkles.

12. A preparation may be made as in 10; but, instead of covering in salt solution, picrocarmine may be used. The slide is placed for some time in the moist chamber until the fibres are stained. This will take a few hours. Then the preparation is irrigated with water, to remove excess of colouring matter, and the water is replaced by glycerine, containing one per cent. of formic acid, a drop of which is placed at one edge of the cover-glass, and allowed to diffuse in. The preparation may then be sealed. The nuclei are red; the contractile substance an orange-colour; the transverse striation well marked.

13. A portion of muscle of a recently-killed frog is torn up and covered in water. The water diffuses through the sarcolemma, and in places raises this from the contractile substance. These blisters of sarcolemma are very commonly seen at the convex side of a bend in the fibre. A clear space is seen outside the striated matter, and bounded externally by a well-defined line, which, by careful focussing, can be seen to have an appreciable thickness. This is the sarcolemma.

If this preparation be irrigated with acetic acid, the nuclei will be well seen, and the fungus protrusions of the contractile substance at the divided ends of the fibres.

14. Tear up in a drop of water the fibres of a muscle which has been kept for some time in alcohol or dilute chromic acid, one-sixth per cent. The tearing should be as fine as possible. It will be found that the fibres readily break into fine threads, some of which are extremely thin (fibrils), but all, down to the very finest, show the transverse striation. By pressing strongly on the cover-glass with the handle of the needle, it will be possible to rupture the sarcolemma of still intact fibres, which will then be seen to break readily into fibrils and groups of fibrils.

15. Tear up fragments of muscle, which have been for twenty-four hours in dilute hydrochloric acid (one strong acid to 250 water). The cleavage into discs may be seen. This does not succeed so constantly as the longitudinal cleavage by alcohol or chromic acid.

16. Make transverse sections of muscle, which has been hardened for ten days in chromic acid and alcohol, and subsequently in alcohol only. Stain the sections in logwood, and mount them in Canada balsam. The blue-stained nuclei will be seen to lie immediately under the sarcolemma. The outline of the fibres is round, or sometimes polygonal, from mutual pressure. The cut surface is covered with dots, the cut ends of the primitive cylinders. Between the fibres is seen the connective tissue, a small quantity about each fibre (endomysium), a larger quantity (perimysium) bounding groups of fibres (secondary bundles).

If a transverse section of frog's muscle be made, the nuclei will appear as angular bodies, situated, not under the sarcolemma, but at all depths in the fibre. The connective tissue is scanty, and the grouping of the fibres into bundles does not exist.

17. Longitudinal and transverse sections of muscles, whose blood-vessels have been injected with gelatine and Prussian blue, and hardened in Müller's fluid or alcohol. Mount in Canada balsam, with or without previous staining in picrocarmine or cochineal. On transverse section most of the vessels are cut across, and appear as blue points between the muscle fibres. On long section the oblong meshes of the vascular network are seen. If the muscles have hardened in the contracted condition the capillaries are tortuous; otherwise they run a straight course.

Subsequently in the tongue admirable views of muscular fibres, cut longitudinally and transversely, will be seen. The sections (16 and 17) may consequently be omitted here.

18. To see the connexion of the muscle with its tendon, the following method, devised by Ranvier, gives the best results. A pithed frog is placed in a litre of water heated to 55° C. Its muscles immediately fall into rigor. It is left about a quarter of an hour in the water, which gradually cools. It is then found that all the tissues are greatly softened. The skin will come off the limbs, if they are simply wiped with a cloth, and the muscles can be readily separated from one another. A small portion of the end of a muscle attached to its tendon is torn up cautiously in water or a drop of picrocarmine. In successful cases the conical or rounded ends of the muscular fibres will be seen retracted within their sarcolemma, which appears empty for a considerable distance, and which can be traced as a continuous membrane, to whose outer surface the tendon is attached. It is difficult to get good preparations; for, on the one hand, the fibre very readily separates altogether from its sarcolemma, and, on the other, the empty portion of this tube is apt to get twisted, or to collapse, so that its continuity cannot be traced. When, however, a successful preparation is got, the appearances are most demonstrative. The lower tendon of the sartorius furnishes good material. A successful preparation, if stained in picrocarmine, may be preserved permanently in glycerine.

19. Muscular fibres may be isolated in their entire length by the following method:—An entire muscle of a recently-killed frog is placed at the bottom of a small beaker, and buried in crystals of chlorate of potash, slightly moistened with distilled water. Over this strong nitric acid is poured. In from about a quarter to half an hour, according to its size, the muscle is removed, and placed in a test tube, half filled with water. It will be found, when the test tube is shaken, that the muscle falls asunder into its fibres, the connective tissue having been dissolved. The transverse striation of the fibres is greatly in-

jured, but the nuclei are visible, and the pointed ends of the fibres can be seen (Kühne).

20. A large water beetle is placed for some days in strong alcohol. One of the legs is then torn off, the horny case of one of the joints opened with sharp scissors, and the contained muscular mass removed. This is stained in logwood, washed, and placed for a few minutes in alcohol. The fibres are then isolated on a slide in a drop of oil of cloves, and mounted in Canada balsam. The details of the transverse striation is very distinct. The fibres frequently have a beaded appearance, being constricted at each thin disc, owing to the close attachment of the sarcolemma to this structure.

21. If the fibres be mounted as in the last preparation, but without having been stained, they are so transparent as to be almost invisible in ordinary light. If, however, they are examined in the dark field of the polarising microscope, they are seen to consist of alternate discs of bright doubly-refracting substance and dark singly-refracting substance.

22. If the muscular tissue of the insect's leg be taken from the living animal, and examined rapidly, without the addition of any fluid, the fibres can be seen in the act of contraction. It will be noticed that the fibre does not contract at once in its entire length, but that the contraction affects only part of the length of the fibre, and travels along this in the form of a wave. This observation should be made with a high power, and is too difficult for a beginner.

CHAPTER VII.

NERVE TISSUE.

Nerves are cords, consisting of a number of fibres (nerve fibres) lying side by side, and bound together by connective tissue of a peculiar character. Some nerves are white, glistening, and opaque, as are most of those belonging to the cerebro-spinal system; others are greyish and semi-transparent, as are the branches of the sympathetic. These differences are due to the fact that the fibres which exist in largest quantity in the cerebro-spinal nerves differ in structure from those which preponderate in the sympathetic. The fibres which give the cerebro-spinal nerves their white appearance are called medullated fibres; those which occur in the sympathetic are called non-medullated.

I. **The medullated nerve fibres** will be first described.

Each medullated fibre is a cylindrical filament, and consists of the following parts. 1. In the axis of the fibre is a thread, which is called the **axis cylinder.** This is believed to be the essential part of the fibre, or that which alone conducts impressions, for in certain parts we find nerve fibres which consist of axis cylinders only, and which are then spoken of as naked axis cylinders. 2. Surrounding the axis cylinder is a layer of a highly refracting substance, of an albumino-fatty composition and semi-fluid consistence. This is called the **white substance of Schwann,** or **myeline,** or **medullary sheath.** It is this which gives the nerve fibre its characteristic microscopic appearance, and it is to this alone that the entire nerve owes its whiteness and opacity. 3. Enclosing the whole is a structureless tubular membrane, quite analogous to the sarcolemma of muscle. This is called the **sheath of Schwann,** after its discoverer, but it is frequently spoken of as the **neurilemma,** and this is the term which will be used here. 4. Oval **nuclei** lie at regular intervals along the fibre. They are situated immediately beneath the neurilemma, and lie in depressions in the myeline. In young animals they are surrounded by protoplasm. Each of these parts has now to be described more particularly.

The **axis cylinder** is generally admitted to have a fibrous structure. It consists of extremely minute fibrils, held together by a homogeneous cementing substance. Where a nerve fibre branches, as commonly occurs, it is believed that the fibrils of the axis cylinder merely separate, some going in one branch, some in the other.

Outside the axis cylinder, and separating it from the myeline, there is supposed by some anatomists to be a sheath, sometimes called the **sheath of Mauthner.** Its existence, although probable, is not absolutely certain. A space filled with albuminous fluid is assumed by some writers to exist in this situation.

The **white substance of Schwann, or myeline,** presents many points for observation. It is not a continuous sheath. Every fibre, at its origin from a nerve cell, wants the myeline sheath for a certain distance, and loses this sheath again before it terminates. At regular intervals along the course of the fibre there occur constrictions, just as if the fibre had had a thread tied around it and drawn sufficiently tight to cut across the myeline, but not to affect the axis cylinder. These are called **Ranvier's constrictions.** At these points the myeline is absent, and the axis cylinder passes through a thickened part of the neurilemma. At each side of the constriction the white substance ends with a rounded extremity, so that the two segments present convex surfaces to each other. These constrictions occur at shorter intervals in fine nerve fibres than in those of greater diameter, and on fibres of the same size they are more closely placed in warm-blooded than in cold-blooded animals. In each fibre, however, they occur with perfect regularity, except near its termination, where the interspaces become shorter.

At the constrictions the axis cylinder can be seen to pass from one segment to the other. Under certain treatment the nerve fibre is marked at this point by a fine transverse line, whose import is not quite certain. At the constrictions the axis cylinder is accessible to various reagents, nitrate of silver, carmine, &c., which cannot reach it at other points, as they cannot penetrate the myeline. It is supposed that the absorption of nourishment by the axis cylinder occurs at the constrictions.

There is an invariable relation between the **nuclei** and Ranvier's constrictions. One, and only one, nucleus occurs in the space between two constrictions, and is always situated exactly midway between the latter. The tract of nerve between two constrictions is supposed by Ranvier to be the equivalent of a cell. It is frequently called an **internode of Ranvier.**

Besides Ranvier's constrictions, other breaks occur, which were described first by Zawerthal, but which are commonly spoken of as **Lanterman's notches.** These are due to the circumstance that the myeline is prone to break into segments which terminate by conical ends, and which are so placed that the sharp end or solid cone of one segment fits into a corresponding hollow cone of the next. When the fibre is viewed from the side, these breaks appear as oblique notches extending on each side of the fibre from the neurilemma to the axis cylinder. These breaks occur without any order, either as regards their number or the direction in which the ends of the cones are placed. Sometimes a considerable tract of nerve is seen quite free from notches, and again they may be so numerous as to make the fibre look like the barbs of a feather. Segments ending with two solid or two hollow cones occur as well as those with a solid cone at one end and a hollow cone at the other. Lanterman's notches have no relation to the nuclei, and they are most numerous when the nerve has been treated with reagents such as osmic acid, which make the myeline brittle, and when the fibres have been submitted to some violence. In all these respects they differ markedly from Ranvier's constrictions; and while there can be no doubt that the latter are not artificial, but exist in the living normal nerve, this cannot be said with at all the same certainty for Lanterman's notches.

It has been already mentioned that the myeline is a very highly refracting substance, and to this probably is owing a peculiar appearance which is perfectly characteristic of a medullated nerve fibre, and occurs in no other fibre in the body. Each nerve fibre is bounded at each side by two parallel lines, or has what is called a double contour. That the inner contour is not caused by the axis cylinder is shown by the fact that where portions of white substance exude from the neurilemma, as they frequently do in preparations, and form globules and irregularly-shaped figures, these figures are bounded by a double contour, just as the nerve fibre is, although, of course, in the extravasated myeline there can be no question of the existence of an axis cylinder. An appearance somewhat similar to that seen in the nerve fibre can be observed in a solid rod of glass looked at against the light. This is observed to have a clear middle part bounded on each side by a broad, dark margin.

In preparations of fresh nerve fibre the normal smooth appearance and even double contour is preserved only for a short time. The outlines soon become irregular, and the surface of the fibre is marked by wavy and twisted lines, the appearance becoming so irregular that it would be impossible to describe it. This is due to a change in the myeline which is commonly spoken of as coagulation, and which consists in the previously homogeneous and fluid white substance dividing into a number of irregularly-shaped masses.

Owing to the great tendency which the myeline has to undergo changes under the influence of reagents, and in the course of these changes to present all kinds of curious figures, many observers have ascribed to it the most complex anatomical structure. There can be little doubt, however, that most, if not all, of these appearances of structure are artificial, and produced only by the agents to whose action the fibre has been submitted. When a nerve fibre is treated successively with cold alcohol, boiling alcohol, and ether, a beautiful network is seen in the place previously occupied by the myeline. This is said to resist pancreatic digestion, and to consist of a horny material, the so-called **neurokeratin.** But it will be noticed that, in the same preparation, this network presents on different fibres the most extreme irregularity, being sometimes coarse and open, at others so firm and close as to appear spongy or granular, and to be seen only with difficulty as a network at all. And while it cannot be demonstrated in a nerve fibre after it has undergone the action of water, it can be made to appear in masses of myeline which have flowed out of the cut end of the neurilemma. It is most probable that the network does not exist in the normal fibre, but is only an artificially-produced appearance, and that it consists of those constituents of the myeline which remain after the removal of the fat by alcohol and ether (Waldstein and Weber).

Myeline swells greatly when it comes into contact with water. When a nerve is torn up in water, the myeline is seen to flow out from the cut ends of the fibres, and along each fibre for a great distance a streaming movement is observed in the white substance as it flows between the axis cylinder and the neurilemma towards the points of exit. These appearances are due to the swelling of the myeline, which is now no longer able to find room within the unyielding neurilemma.

Owing to its fatty constituents, the myeline becomes black when treated with osmic acid. This reagent also coagulates the myeline, but preserves its smooth outlines, and prevents its undergoing change by the action of water and other fluids. Hence, for the study of many points in the anatomy of the nerves, osmic acid is an invaluable aid. A fibre treated with osmic acid appears with dark margins and a less dark central part, the thickness of myeline through which the light has to pass being greater at the margins than in the centre. The axis cylinder is unstained by osmic acid. All interruptions in the myeline are consequently very distinct in osmic acid nerves.

In young nerve fibres a thin layer of protoplasm extends between the neurilemma and the myeline for a considerable distance at each side of the nucleus, if not over the whole internode. In the fibres of adult animals no protoplasm can be demonstrated, except close to the nucleus.

The **neurilemma** is not certainly known to have any structure. At each of Ranvier's constrictions it presents an annular thickening, through which the axis cylinder passes.

Nerve fibres vary a good deal in thickness. It has been found that the longer the fibre is from its origin to its termination, the greater is its diameter (Schwalbe).

II. **The Non-medullated Nerve Fibres:**

The non-medullated nerve fibres, or the **fibres of Remak,** occur most abundantly in the branches of the sympathetic, but also, in less number, in the cerebro-spinal nerves. This is owing to the numerous communications between the sympathetic and cerebro-spinal systems. To the same cause is due the presence, in considerable numbers, of medullated fibres in the trunk and branches of the sympathetic.

The Remakian fibres appear as threads of various thickness. They present a more or less distinct longitudinal striation, indicating that they are composed of **fibrils,** which are held together by cementing substance. The fibres are surrounded by a **neurilemma.** Ranvier has pointed out the very remarkable fact that the non-medullated fibres do not run an isolated course, as do the medullated fibres, but that they branch and anastomose freely with one another, and in fact form a plexus with narrow meshes, elongated in the direction of the trunk of the nerve. Situated under the neurilemma and generally at the points of division of the fibres are oval **nuclei,** surrounded by a small quantity of protoplasm. The nuclei are more numerous and not so regularly arranged as in the medullated fibres.

The non-medullated fibres have, of course, no double outline, and are not blackened by osmic acid.

III. The **white substance of the brain and spinal cord** consists mainly of nerve fibres, which are distinguished by the absence of a neurilemma, while they possess an axis cylinder and white substance of Schwann. Hence these fibres want firmness, and cannot be isolated for any length, in consequence of their softness. They appear when torn up as short, varicose, double-contoured threads, or as quite irregular fragments, although when in their natural state they are cylindrical fibres, like those of the medullated nerves. It is not known that they possess either constrictions or nuclei.

At their peripheral extremities the nerve fibres lose their white substance, and the axis cylinders terminate either in peculiar structures, which are called **end organs**, or, becoming reduced by repeated divisions to extreme fineness, they form plexuses, and terminate either in **networks**, or by **free ends**. These terminal axis cylinders are 'naked'; that is, they have no coverings, but have lost not only their myeline, but also their neurilemma and their connective tissue sheaths.

Before the medullated fibres lose their myeline they commonly branch pretty freely. One fibre may divide into two or more, the division invariably occurring at one of Ranvier's constrictions. Since near the termination of the fibres the branching is frequent, the intervals between the constrictions are here necessarily much shorter than along the course of the fibre, but up to the last each internode possesses a nucleus.

The nerve fibres, when they do not run singly, are always arranged in bundles. The smaller nerves consist of only a single bundle, but the large trunks are composed of several, and these bundles are the threads, of which with the naked eye the nerves are seen to be composed. The bundles are held together by connective tissue, which does not differ materially from the areolar tissue of neighbouring parts, with which it is continuous. It consists of fibrous and elastic tissue, in which are situated flattened cells, fat, and very commonly large, coarsely granular plasma cells. This connective tissue, which surrounds the whole nerve trunk, and passes in between its bundles, is called **Epineurium.**

Surrounding each bundle of fibres is a peculiar sheath, which is composed of a number of lamellæ, and is called **Perineurium**. Each lamella consists of a layer of connective tissue, with elastic fibres, and is covered by flat cells, resembling an endothelium. The lamellæ frequently communicated with one another by oblique processes, and in the larger bundles the lamellar sheath commonly sends septa, consisting of several lamellæ, into the bundle, which is in this way divided into secondary or tertiary bundles. The bundles of which a nerve trunk is composed are of various sizes, and generally the larger the bundle the thicker the perineural sheath. As the bundles become smaller by division the perineurium becomes reduced to fewer and fewer lamellæ, until in the smallest bundles it consists only of a single layer of homogeneous connective tissue, with flattened cells on its inner surface. It lies at some little distance from the nerve, and is continued even on the individual fibres up to near their termination. This sheath, consisting of only a single layer, is called **the Sheath of Henle.**

The spaces between the lamellæ of the perineurium can be injected from the subdural and subarachnoid cavities of the brain and cord, and must be considered as lymphatic channels.

Inside the perineurium, and between the nerve fibres, is a very delicate tissue, called the **Endoneurium**. It consists of bundles of extremely fine connective tissue, which run parallel to the direction of the nerve fibres, and which have at intervals flat connective tissue cells lying on their exterior. There is neither elastic tissue nor fat in the endoneurium.

Blood-vessels penetrate the perineurium, and form among the nerve fibres a network of capillaries, with elongated meshes. The vascularity of nerve trunks, however, is not very great.

We shall here describe the way in which the nerves terminate in the three varieties of muscular tissue. Other nerve endings will be noticed in subsequent chapters.

I. In the smooth muscular tissue the nerves form a plexus, from which branches come off which form a finer plexus, and finally a terminal plexus is formed, consisting of non-medullated fibres, which run between the muscle cells composing the bundles of the tissue. From these fibres minute filaments are detached, and terminate on the surface of the muscle cells by slightly swollen ends (*taches motrices*), which in profile view appear slightly conical, but seen from the surface, often present a number of little lobes. The nerves, distributed to smooth muscle, are commonly derived from the sympathetic, and the plexuses have in connexion with them groups of ganglionic cells.

II. The exact mode in which the nerves terminate in the tissue of the myocardium cannot yet be considered as certainly known. Numerous ganglia occur on the course of the nerves in the auricles, and in the neighbourhood of the auriculo-ventricular junction; but nerve cells have not been demonstrated in the walls of the ventricles. The nerves ultimately form a network of extremely minute non-medullated fibres, which corresponds somewhat to the shape and size of the cells of which the muscular tissue is composed. From Ranvier's observations it would appear that the muscle cells do not lie in the meshes of this network, but that the threads composing it pass through the axis of the muscle cells, which are thus, as it were, strung on the nerve fibres as beads are on a string. These observations require confirmation.

A more recent investigation would show that from a very abundant plexus, terminal fibres come off, and end in the muscle cells by little swellings, resembling closely the *taches motrices* of the smooth muscle. Each muscle cell receives one nerve fibre (Openchowski).

III. In the striated muscles three kinds of nerve terminations are known, and it is probable that others exist.

1. In insects the nerves are all non-medullated. One or more fibres, contained in a common sheath, come to terminate in the muscle fibre. The nerve sheath becomes continuous with the sarcolemma, spreading out over a conical eminence (eminence of Doyère) of granular matter, which contains several nuclei, and rests by its base on the striated muscular substance. Into the apex of this cone the nerve fibres pass, and breaking up into minute fibrils, are lost to view. It has been affirmed recently that the fibrils terminate by becoming continuous with the thin discs of the contractile substance, or Krause's

membrane (Föttinger); but this statement still wants confirmation. Each muscular fibre possesses several terminal eminences.

2. In frogs and other tailless batrachians the medullated nerve fibres, as they approach their termination in the muscles, branch very freely, the divisions occurring always at a constriction of Ranvier. Each fibre terminates finally by dividing into a number of branches (*buisson terminale*), which ramify up and down on the surface of the primitive muscular bundle which the nerve supplies. The primary branches of this ramification retain their myeline, and are surrounded by the sheath of Henle. This latter becomes continuous with the sarcolemma, and beyond this point of junction the branches of the nerve are non-medullated, and lie in immediate apposition with the contractile substance beneath the sarcolemma. At what point the neurilemma of the nerve fibre is lost is not certain. Associated with the terminal ramifications of the nerve are oval nuclei.

Transitional forms between the typical ramification of the amphibia and the end plates of the higher animals have been recently described as occurring in frogs (Bremer), and are readily seen in good preparations.

3. In the muscles of reptiles, fishes, birds, and mammals the termination of the nerves is different from that in amphibia. Here the nerve fibre, after repeated divisions, still retaining its myeline and sheath of Henle, approaches its termination. The sheath of Henle becomes continuous with the sarcolemma. The myeline disappears, and the axis cylinder breaks up into a bunch of processes. These vary in thickness from point to point, and give off numerous secondary branches, which correspond in structure and arrangement to the primary divisions. They all terminate by slightly rounded ends. The whole forms a very elegant figure, of a round or oval shape. It lies beneath the sarcolemma, and is sur-

rounded by a variable quantity of granular matter, containing nuclei. Whether it lies in immediate contact with the contractile substance, or is separated from it by some of this granular matter, is a subject on which different opinions are held. Besides the nuclei of the granular matter, there are others, which belong to the nervous ramification itself, and which are probably the nuclei of the nerve fibres; and, again, others which are situated on the deep surface of the expansion of the sarcolemma and sheath of Henle, which covers the whole terminal structure. The fate of the neurilemma is here, as in the case of the frog, uncertain. This form of nerve termination is called a motor end-plate. Ranvier proposes *arborisation terminale* as a more suitable designation.

In the frog and other mammals two nerve fibres frequently take part in the formation of one terminal organ. The terminal organs on neighbouring muscle fibres are sometimes united by nerve twigs. This is not uncommon in the tongue of the frog and lizard (Bremer. Ranvier).

Besides the above terminations, which are all those of motor nerves, another has recently been desccibed under the name of terminal umbel (Enddolde), from its resemblance to the inflorescence of that name. It is in connexion with nerves which, from their fineness and other characters, are supposed to be sensitive (Bremer). The fibre terminates under the sarcolemma by breaking into a number of diverging branches, which form the umbel-like figure, and terminate each in a little rounded enlargement.

Ganglia:

Besides the **ganglionic cells,** which form the most important constituent of the grey matter of the brain and cord, numerous nerve cells exist in the peripheral parts of the body in connexion with the nerve trunks. Some are in connexion with the branches of the sympathetic, others with those of the cerebro-spinal, nerves. The cells sometimes lie singly or in groups of only microscopic size; in other cases they exist in large numbers, and form masses of considerable dimensions.

In the larger ganglia the cells lie in rows and groups of various shapes between the bundles of nerve fibres which traverse them.

The smaller ganglia may lie altogether at one side of a nerve, or may be enclosed between its fibres.

Ganglionic cells are of various dimensions, but are usually large. They have a finely granular protoplasm, and a large, clear nucleus, with very distinct nucleolus. Their shape is various—oval, pyriform, round, or stellate.

Each cell is commonly enclosed in a sheath, which is composed of flattened endothelioid plates. There is sometimes an evident space between the cell and its sheath, supposed to be of a lymphatic nature. Two or more cells may lie in a common sheath.

Ganglionic cells are in connexion with nerve fibres by their processes—in fact, the nerve fibres are only the greatly elongated processes of the ganglionic cells either of the brain and cord or of the peripheral ganglia.

Ganglionic cells are commonly divided, according to the number of processes which they possess, into—1. Apolar, 2. Unipolar, 3. Bipolar, and 4. Multipolar.

Apolar cells probably do not exist except as imperfectly developed structures. In the course of microscopic manipulation a cell may readily have its processes torn off, and may thus come to appear as if it were apolar.

Unipolar cells are most common in all the ganglia of the cerebro-spinal nerves. In the ganglia on the posterior roots of the spinal nerves, the Gasserian ganglion, the ganglia on the roots of the pneumogastric and glossopharyngeal, the geniculate ganglion of the facial and the ganglion of the auditory nerve, each cell sends off a single process. This soon becomes clothed with white substance, and joins one of the nerve fibres, which traverse the ganglion, forming with it a sort of T-shaped figure. The junction occurs at a Ranvier's constriction (Ranvier. Retzius).

This arrangement explains the fact that the number of fibres in a spinal nerve root experiences neither increase nor diminution in traversing the ganglion. If the processes of the cells of the latter passed in either direction, the number of fibres at the proximal side of the ganglion should differ from that at the distal side.

There are in many places unipolar cells whose connexion with nerve fibres is direct, not by a T-shaped junction.

Bipolar cells present themselves under different forms. Sometimes they are oval, the processes passing off from opposite ends. The cell then appears as a swelling of the axis cylinder on the course of the nerve fibre. The neurilemma passes over the cell, but the myeline is interrupted. Cells of this kind are common in fishes, and occur in the spiral ganglion of the cochlea of mammals. A very remarkable variety of bipolar cells exists in the sympathetic system of frogs, and less abundantly in other animals. The cell is pyriform, and the two processes come off close together. One process runs a straight course, while the other is twisted around it in a spiral. These cells were discovered almost simultaneously by Beale and Arnold, and are consequently called the Beale-Arnold cells.

Multipolar cells are very common, and occur abundantly in the sympathetic ganglia, to which seem to belong the otic, sphenopalatine, submaxillary, and ciliary ganglia (Retzius).

In all ganglionic cells, whether central or peripheral, the cell substance, more particularly at its outer part, presents a fibrous appearance, and it can often be seen that the fibres pass into the processes of the cell, and become continuous with the axis cylinders of the attached nerve fibres.

1. When fibres of a nerve bundle are to be isolated, it is necessary to divide the perineural sheath. When this is accomplished, the fibres will separate from one another with facility,

since the fibres of the endoneurium run parallel to the nerve fibres, and consequently do not hold the latter together. The larger bundles of a nerve trunk are easily seen, and the perineurium may be divided with fine scissors or a sharp scalpel. A more ready way, however, is to fix the piece of nerve, which should not be more than a few millimetres in length, at its end with one needle, and with another, starting from the same point, to tear through the nerve bundle in its length. It will then be easy with a few touches of the needle to separate a sufficient number of fibres. This should be done on a dry slide, keeping the preparation moist with the breath. Then a drop of salt solution is placed on the cover-glass, and the preparation covered.

The nerve fibres will be seen. They are of very variable diameter. In many the coagulation phenomena will be visible, and the fibres will present irregular markings on their surface, due to the alterations of the myeline. Others will be altered in this way only in the neighbourhood of their cut ends, and in the rest of their course will show the normal smooth outlines and double contour. Ranvier's constrictions will be very distinct, and it will be seen that the double outline is interrupted at these points. Lanterman's notches also will be seen more or less clearly. In some points the nuclei may be discovered. They will be seen only if they present themselves in profile, when the double outline on one side will be seen to be deflected towards the axis of the fibre, leaving a space between its exterior and the neurilemma. On careful examination this space will be seen to contain a very finely granular substance, which is the nucleus and surrounding protoplasm. After a time, coagulation changes will invade all the fibres.

2. A preparation of fresh nerve fibres is made, and examined in distilled water. From the cut ends of the fibres the myeline will be seen to flow out in a rapid current, forming irregularly-shaped figures, with double outlines, and often concentric markings. In the fibre, near the point of efflux, the myeline will be seen to present a streaky or granular appearance, and to consist of several concentric layers, which move with different rapidity towards the end of the fibre; but further from the cut end it will have its smooth contour, and will be seen to move altogether. Lanterman's notches move with the myeline, and in favourable objects this can be seen to force its way through the narrow points of Ranvier's constrictions. In certain points of the fibre the axis cylinder will be very distinct, and the whole appearance of the preparation demonstrates the presence of the firm neurilemma, which holds in the swelling myeline, and allows it to protrude only at the cut end of the fibre.

3. A small piece of fresh nerve is placed in one per cent. osmic acid for twenty-four hours. If the nerve be thin, as the sciatic of the frog, it may be simply divided transversely into segments;

but if thicker, it must be separated longitudinally into its bundles, as osmic acid does not penetrate. A few minutes after being placed in the osmic acid, the nerve becomes quite black. After twenty-four hours the nerve is washed in water, and the fibres are isolated. Since they are very brittle, the best way to proceed is to place the piece of nerve in a saucer of water, and to divide its perineurium under the fluid. Some of the fibres will float out, and will be easily seen as fine black threads. A clean slide is then dipped into the water, and the fibres floated on to its surface. The slide is then taken out of the water, dried, the fluid removed from about the fibres with filter paper until they are sufficiently dry to cohere slightly to the slide, a small drop of glycerine placed on the cover-glass, and this placed on the preparation.

The fibres are black, with a broad, dark margin, and a lighter centre. Ranvier's constrictions are very distinct, as the blackness ceases at the narrow part. Across the bright interval the axis cylinder can be seen to extend, and, particularly in frog's nerves, at the point of constriction there will be noticed a fine granular line extending transversely across the axis cylinder, and projecting beyond it at both sides. This has been variously explained. It is probably a thickening of the neurilemma through which the axis cylinder passes. Lanterman's notches are distinct. The nuclei appear as colourless depressions in the side of the fibre, filled by a finely granular substance.

Cross sections of a nerve stained in osmic acid will show the myeline of each fibre as a black ring enclosing the colourless axis cylinder.

After a prolonged stay in osmic acid, nerve fibres cannot be stained in picrocarmine. If, however, the fresh fibres be separated in a drop of osmic acid solution, and immediately washed in distilled water, they may be stained in picrocarmine, and mounted in glycerine. The nuclei and, in favourable preparations, the axis cylinders are stained red. Also the nuclei of the endoneural cells, which, with their fine fibres, may be seen in places lying between the nerve fibres. The myeline of the latter is black. In places the axis cylinder may be seen projecting beyond the broken end of a fibre, or extending across a break in the myeline. These fractures in the myeline are transverse, with sharp edges; neither rounded, like Ranvier's constriction, nor oblique, like Lanteman's notches. A little experience and care will enable the diagnosis to be always readily made.

Osmic acid preparations may be mounted permanently.

4. A piece of the sciatic nerve of a frog is placed for twenty-four hours in alcohol, in a stoppered bottle, then boiled for ten minutes in alcohol, and finally placed for twenty-four hours in ether. The fibres, isolated with needles, may be examined in water; or stained with eosine, logwood, or aniline violet, and mounted permanently in glycerine. The place of the white

substance will be seen to be occupied by a beautiful and regular network. The axis cylinder will be seen in the centre of the fibre.

5. A piece of fresh nerve is torn up in a drop of half per cent. solution of nitrate of silver. The silver solution is washed away with distilled water, and a drop of glycerine and a cover-glass applied. After the preparation has been for some time exposed to the light it becomes brown. Then at each constriction of Ranvier the granular transverse line, described above (3), and a short portion of the axis cylinder at each side of it, are stained brown or black, the whole figure being that of a rectangular cross, with short arms. This shows that the axis cylinders are accessible to the reagent only at Ranvier's constrictions. A brown transverse striation is sometimes seen in places on the axis cylinder. The explanation of this very inconstant appearance is doubtful.

6. A piece of the sympathetic nerve of a rabbit is torn up in a drop of osmic acid solution, washed, stained in picrocarmine, and mounted in glycerine, as described in 3. Numerous medullated nerve fibres are seen, mostly of very small diameter. In these the nuclei are relatively large, and as they cause a bulging of the fibres, they are very readily seen. Besides, there are large numbers of non-medullated fibres, which, if the preparation be successful, appear as streaky bands, dividing and anastomosing, so as to form a network, with narrow and elongated meshes. They have a yellowish colour from the picrocarmine, but are not blackened by the osmic acid. They present numerous oval nuclei, stained red, and occurring commonly at the points of division of the fibres.

7. A small fragment of the white substance of the brain or cord is torn up in a drop of salt solution, and covered, the cover-glass being slightly pressed, so as to flatten the tissue. Numerous short varicose threads are seen. They have a double contour, which is generally continued over their extremities, the myeline having coalesced over the ends of the axis cylinders. Irregular-shaped figures, also with double contours, and consisting of masses of myeline, are present.

8. A preparation of the cornea of a rabbit or frog which has been stained in chloride of gold should be examined. The fine varicose threads stained black are terminal nerve filaments, or naked axis cylinders. A detailed description of the nerves of the cornea will be given subsequently.

9. The branching of medullated nerve fibres may be beautifully seen in the muscles of the thigh of a frog. Fragments of the fresh muscle torn up in salt solution give good results; but to make permanent preparations the best method is as follows: With a hypodermic syringe, which should have a gold cannula, a few drops of a one per cent. osmic acid solution are injected into the substance of one of the thigh muscles of a recently-killed frog.

After a few minutes pieces are cut with scissors from the interior of the muscle, and are gently torn up in water on the slide. The preparation should be examined from time to time with a low power, and when a good piece is got, it should be covered with a large drop of picrocarmine, and placed in the moist chamber for twenty-four hours. Then the picrocarmine is removed by a drop of water, and a drop of glacial acetic acid is applied. The excess of this is carefully taken away, and the preparation finally mounted in glycerine. In successful preparations the frequent branching of the nerve fibres is seen, and it will be observed that the division always occurs at a constriction of Ranvier. These constrictions occur at short intervals, and the reddened nuclei can be well seen, one midway between every two constrictions. Surrounding the nerve fibres at some little distance will be seen the sheath of Henle, appearing as a fine line on each side of the fibre, with spindle-shaped nuclei placed at intervals on its inner surface. The muscular fibres will be of an orange colour, the nuclei red.

10. A piece of the sciatic nerve of a dog, or of one of the larger nerves of man, is hardened for ten days in quarter per cent. chromic acid, and subsequently in alcohol. Before being removed from the body the piece of nerve should be tied at both ends to a piece of wood, so as to prevent shrinking and the displacement of the fibres. Transverse sections through the whole nerve are made, and stained in logwood, cochineal, or picrocarmine, and mounted in Canada balsam. With a very low power each nerve-bundle appears as a more or less circular figure, surrounded by a deeply-stained ring (perineurium). Connecting these bundles together is loose areolar tissue (epineurium), in which large blood-vessels, and possibly fat, may be seen. With a higher power, in the interior of each bundle will be seen the cross sections of the nerve fibres, each appearing as a circle (neurilemma), with a stained dot (axis cylinder) in its centre, from which it is separated by a colourless interval (myeline). Between the nerve fibres is seen a faintly dotted or homogeneous matter, with round or oval nuclei here and there (endoneurium). Blood-vessels will also be seen cut across in the larger bundles. Surrounding each bundle is the perineurium, consisting of concentric lamellæ, with intervals between, and having on their surfaces spindle-shaped nuclei, which are the profile view of the flattened nuclei of the endothelium. Processes from the perineurium often pass across the larger bundles, dividing them into secondary or tertiary fasciculi. Outside the perineurium is seen the areolar tissue of the epineurium. Very beautiful preparations may be got by staining a cross section of a nerve first in logwood, then in an alcoholic solution of eosine, and mounting in Canada balsam. The axis cylinders are red; the nuclei of the endoneurium and of the perineurium blue the lamellæ of the latter red.

11. If a very small nerve, consisting of a single bundle, and whose perineurium is composed of only one or a few lamellæ, be stained with nitrate of silver, and mounted in glycerine, a beautiful and regular endothelial marking is visible on its surface. This is due to the endothelial cells, which line the perineural lamella. At the same time the crosses, 5, on the nerve fibres may be seen.

Suitable nerves for this observation are the lateral branches of the intercostal nerves of rats or mice (Ranvier). The skin is divided in the middle line in front, and drawn away from the body at each side. The fine nerves will be seen perforating the intercostal spaces, and extending to the skin. They should be cleared with a needle from adhering connective tissue, and while the skin is held away from the body, so as to keep the nerves on the stretch, the nitrate of silver is dropped over them from a pipette. They are then carefully removed, and placed in a saucer of water, floated on to the slide, mounted in glycerine, and exposed to the light until they are brown.

12. A sympathetic ganglion of rabbit, dog, or man, and a spinal ganglion, hardened in Müller's fluid for a fortnight, and then in alcohol, are cut. The sections should be longitudinal and transverse, stained in logwood, and mounted in Canada balsam. The cells are seen, lying in rows and groups, with the nerve fibres between. Each cell has a large nucleus, and lies in a sheath, in which flattened nuclei can be seen. The cell will probably have shrunk, so as no longer to fill completely its sheath, and shallow depressions may be seen on its surface, corresponding to the nuclei of the endothelial cells of its capsule. The processes of the cells will not be seen in such sections.

13. A sympathetic, or spinal, or Gasserian ganglion, divided into small pieces, is placed in Müller's fluid and water, equal parts, for twenty-four hours; then torn up in water or glycerine. Or, still better, with a hypodermic syringe an injection of one per cent. osmic acid is made into the substance of the fresh ganglion, which is then placed in water, and cut into small pieces, which are torn up in water or glycerine. The isolated cells will be seen, some still lying in their capsules (the cellular structure of which is very distinct), some naked. In fortunate preparations the processes of the cells may be seen here and there. In the sympathetic ganglia several processes may adhere to one cell, but in the Gasserian and spinal ganglia there will never be more than one process. It is possible, but not very likely, that in osmic acid preparations this process may be followed to its T-shaped junction with a nerve fibre. Divisions of nerve fibres in spinal ganglia are, however, commonly seen in osmic acid preparations. In the sympathetic ganglia of the rabbit most of the nerve-cells have two nuclei.

It will be noticed in the ganglia which have been for some time in Müller's fluid, that the myeline of the nerve fibres has become brittle and readily crumbles away, leaving the axis cylinders exposed for a considerable distance. Torn-up preparations of ganglia, if successful, may be mounted permanently in glycerine.

Preparations of ganglion cells with spiral fibres, and of peripheral ganglia should be examined. They are rather difficult to make. Subsequently, in examining sections of the different organs, groups of ganglion cells will be frequently met with.

The termination of the nerves in muscle are best seen in preparations stained with chloride of gold. The method is too difficult for beginners. Preparations, however, should be examined.

Directions for staining with chloride of gold will be given in the Appendix.

CHAPTER VIII.

BLOOD-VESSELS.

THE blood-vessels consist of the heart, arteries, capillaries, and veins. The muscular tissue of the heart has been already described. The heart is covered externally by the **pericardium,** a serous membrane composed of fibrous and elastic tissue, covered by an endothelium whose cells are very irregular in shape. Under the pericardium there is found in places a considerable quantity of fat.

The **endocardium** lines the interior of the cavities of the heart. It is continuous with the internal coat of the blood-vessels. It consists of a layer of spindle-shaped endothelial cells, under which is a tissue of a fibrous appearance, containing flattened cells, and mixed with elastic tissue and bundles of smooth muscular fibres. Below this is ordinary connective tissue, in which ramify vessels and nerves. The **valves** are composed of fibrous tissue, with flattened cells, and an elastic network. They contain numerous blood-vessels. The **cordæ tendineæ** are formed of fibrous tissue, and are covered by endothelium, as are the valves. The tissue of the heart is supplied abundantly with blood-vessels and nerves. The **lymphatics,** which originate in the split-like spaces between the muscular bundles, are continued into a close plexus of vessels under the pericardium.

I. **Arteries:**

The structure of the arteries differs greatly according to their size, but they all have **three coats,** which are called the internal, middle, and external, or tunica intima, tunica media, and tunica adventitia.

In the **very large arteries,** as the aorta, iliacs, carotids, **the internal coat** consists of a layer of spindle-shaped endothelial cells, the long axis of the cells being in the direction of the axis of the tube. Under this is a tissue of a fibrous structure, mixed with fine elastic networks, and containing branched and flattened cells.

The middle coat consists mainly of elastic tissue, in the form of numerous laminæ, perforated with holes (fenestrated membranes), and arranged concentrically around the vessel. These laminæ communicate freely with one another by fibres and bands passing from one to the other, so that the whole forms a coarse network, with wide meshes. The internal and the external lamellæ are stronger than those lying between.

The interspaces in the elastic tissue are filled up by a homogeneous or faintly fibrous connective tissue, in which are embedded stunted smooth muscle-cells, whose direction is at right angles to the length of the vessel. In the aorta and some other arteries muscular fibres occur, which are arranged longitudinally and obliquely.

The external coat consists of ordinary connective tissue, in which is contained an abundant elastic network.

In **middle-sized arteries,** from the femoral and brachial downwards, the **internal coat** is the same in structure as in the large arteries, but it is thinner. It is separated from the middle coat by a perforated elastic membrane, called the **internal elastic lamina.** The **middle coat** is formed almost entirely of smooth muscular fibres arranged transversely. Between them is a certain quantity of connective tissue and an elastic network, but these diminish as the arteries become smaller, and instead of forming the great bulk of the middle coat, as they do in the main trunks, they are here quite subordinate to the muscular element.

An elastic membrane, the **external elastic**

lamina, separates the middle from the **external coat,** which consists of areolar and elastic tissue.

Small Arteries.—As the arteries become smaller, all their coats diminish in thickness, retaining, however, the same constitution as before. When we reach the **very small arteries,** or arterioles, we find the **internal coat** reduced to the endothelium and a thin subjacent elastic membrane or network: the **middle coat** consists of a single layer of smooth muscular fibres, which are arranged so that their nuclei do not follow each other in one straight line, but run in a spiral around the tube: the **external coat** is composed of a small quantity of connective tissue and of elastic fibres.

II. The **capillaries** have a simpler structure than any other part of the vascular system. They consist of a single layer of elongated endothelial cells, cemented edge to edge, so as to form a tube. The existence outside this of a homogeneous membrane is doubtful. In some parts connective tissue cells form a sort of outer coat to the capillaries, and have been named perithelium. The capillaries anastomose freely with one another, so as to form networks, loops, &c., whose shape and arrangement differ in the different tissues and organs.

III. **The veins** are much thinner, and generally more capacious than the corresponding arteries, from which they differ materially in the structure of their coats. There is also very great variety in the structure of different veins.

It is usual to describe veins as having, like arteries, three coats, but since no sharp distinction exists between the two outer tunics, it is much better to follow Ranvier in describing the veins as consisting of only **two coats**.

The endothelium is composed of cells, which are shorter and broader than those in the arteries. In the **small veins** the cells rest on a scanty elastic network, outside which is connective tissue, in which the muscle cells are few and scattered, and do not form a continuous layer, as in the arteries.

In the **larger veins** the endothelium rests on a fibrous layer, with flat cells, similar to that which forms the internal coat of the arteries, but thinner and containing less elastic tissue. Outside this is an elastic network, corresponding to the internal elastic lamina of the arteries, and then follows the outer coat, whose composition varies. Its basis consists of elastic networks and connective tissue. In this is embedded smooth muscular tissue, whose fibres are arranged circularly, longitudinally, or both. It is not uncommon to find a layer of circular muscular fibres immediately under the intima, and numerous longitudinal bundles of fibres further out in the connective tissue. In some veins longitudinal fibres only occur; in others transverse only; in others a transverse layer between two longitudinal; while some veins are altogether wanting in muscular tissue. The veins of the lower extremities have thicker and more muscular coats than those of the upper extremity and head and neck. In the pulmonary veins transversely striated muscular tissue occurs, continued from that which forms the wall of the auricle. The **valves** in the veins are formed of a duplicature of the internal coat.

The larger arteries and veins are themselves supplied with blood-vessels, the **vasa vasorum**. These run in the adventitious coat, but do not penetrate the intima or media. **Lymphatics** also are present in the external coat. The blood-vessels are accompanied by **nerves**, which form plexuses in their walls, and which terminate in the muscular fibres of the middle coat. On the walls of the capillaries the nerves terminate by small knob-like swellings (Bremer).

1. The smaller blood-vessels can be examined as a whole, but sections have to be made through the coats of the larger arteries and veins.

The omentum is removed from a recently-killed rabbit, and placed for some days in Müller's fluid, and then well washed in water. Pieces containing vessels which can be seen are cut out, stained in logwood or picrocarmine, and mounted in glycerine, following the directions given previously for putting up membranous structures. The capillaries appear to be bounded on each side by a fine line, on which occur at intervals spindle-shaped nuclei, with their long axis parallel to that of the vessel. These are the nuclei of the endothelial cells. Tracing the tube towards the arterial side, it is seen to gain a layer of transversely-disposed muscular fibres, whose elongated nuclei lie at right angles to those of the endothelium. On focussing down, so as to see the edge of the artery distinctly, the muscular nuclei appear as round points, sometimes at one side of the artery, sometimes at the other. This is due to the fact that the nuclei are not disposed in one line, but form a spiral around the vessel. Inside the muscular coat a shining membrane, wrinkled longitudinally, will be seen, the internal elastic lamina. Outside all is a layer of connective tissue, whose bundles run for the most part longitudinally, and whose outer limit is badly defined.

The veins show the oval endothelial nuclei distinctly; no distinct internal elastic lamina; few and scattered muscle cells, but a well-developed external coat.

In many places the veins and arteries run side by side, so that they can both be brought into view together, and their structure compared. Communications between arteries and veins by a dense and complicated network of capillaries occur in many places in the omentum of the rabbit. The meshes of the network are occupied by numerous cells, resembling lymph corpuscles. Such places appear in the fresh membrane as whitish semi-opaque patches.

2. The blood-vessels of a recently-killed animal are washed out with a current of distilled water, and then injected with a half per cent. solution of nitrate of silver, which, after it has remained for some minutes in the vessels, is washed out with water. Transparent parts, as the mesentery, omentum, &c., are removed, and exposed to the light in dilute alcohol. The vessels soon appear as brown streaks. Portions may be then cut out, and mounted in glycerine. If it is desired to see the nuclei, the piece of membrane must be stained in logwood or picrocarmine after the silver has acted.

The endothelium of the vessels is marked out by fine black, generally sinuous lines. In the arteries the cells appear as very narrow, elongated figures. Their narrowness is in great part due to the contraction of the muscular coat under the stimulus of the injection. In the veins the cells are broader and shorter; in the capillaries irregular, one cell frequently reaching around the whole circumference of the tube. Between the larger cells smaller figures are sometimes seen, which have no nuclei, and whose nature and import are doubtful. The intercellular lines present in the capillaries and small veins thickenings here and there, the so-called stomata and stigmata. It is probable that they are merely precipitates of silver in little accumulations of the albuminous cement-substance. In the arteries, besides the endothelial markings, the outlines of the transversely-arranged muscular fibres are seen as black lines running across the vessel.

3. Transverse sections of small arteries and veins are constantly met with in the examinations of the different organs. The artery presents internally a ring of circular or oval dots, the cross section of the endothelial nuclei. The appearance of the endothelium differs according as the vessel is distended or contracted. In the former case the cells are extremely thin, and the nuclei flattened; but when the tube is contracted the cells are much thicker (Renaut), the nuclei are round, and frequently project into the lumen of the vessel. Outside the endothelium is a shining, homogeneous line, which usually surrounds the vessel in a wavy course, giving the lumen a stellate form. This is the internal elastic lamina. External to this is a thick layer, in which spindle-shaped nuclei can be seen. These are the nuclei of the muscular fibres of the middle coat, and, together with the internal elastic lamina, are most characteristic of an artery. The external coat consists of connective tissue, and passes insensibly into the surrounding parts.

The vein has a round or oval lumen, bounding which is the endothelium, whose nuclei are distinct. There is no internal elastic lamina, but a fine elastic network is continued into the parts about. In this network, besides connective tissue, there occur, in variable number, scattered muscular fibres, recognisable by their nuclei, which appear round or spindle-shaped according as the fibres are cut across or longitudinally. In many cases the veins appear as little more than a space in the tissue, bounded by a ring of endothelial nuclei. The thinness and want of definition of the walls, with the absence of internal elastic lamina and compact muscular coat, serve always to distinguish a vein from an artery in sections.

4. Owing to the large quantity of elastic tissue in their walls, it is difficult to cut sections of the larger vessels, as these sections must be extremely thin. They may be cut in the freezing microtome, or the vessel may be laid open, and a piece of it pinned out on a cork and dried. Very thin sections can then be made, if the piece of dried tissue is wedged into a slit in a piece of cork. The dry shavings of the vessel may be allowed to swell on the slide in a drop of picrocarmine, and, when stained, they may be washed in water, and mounted in glycerine containing one per cent. of formic acid (Ranvier). The direction of the section should be accurately longitudinal or transverse. Longitudinal sections are most instructive.

Longitudinal section of a middle-sized artery, such as the human radial. The endothelium has probably been destroyed in making the preparation. The internal coat appears as a streaky layer, with long, spindle-shaped nuclei, the profile view of the nuclei of the flat cells of the intima. A well-defined elastic lamina, stained yellow by the picrocarmine, separates the intima from the thick middle coat, which consists almost entirely of muscular fibres, all appearing here in cross section. The muscular tissue is bounded externally by a well-defined elastic layer, the external elastic lamina, beyond which is the connective tissue and elastic networks of the adventitia.

In a long section of a large artery, such as the human aorta, the internal coat is thicker, and contains more elastic tissue, but is otherwise similar to that of the radial. The middle coat appears as a number of thick, shining lines running parallel to one another, and which are sections of the elastic laminæ. These lines are interrupted here and there where holes occur in the laminæ, and communicate freely with one another by thick elastic fibres. The interstices of the elastic framework are occupied by a faintly stained, homogeneous connective tissue, in which are embedded smooth muscular fibres, which are mostly cut across, and appear as round figures. In the aorta of the ox the muscular fibres lie not only between the elastic laminæ, but in places the elastic membranes are interrupted by masses of muscular tissue, the direction of whose fibres is commonly longitudinal or oblique, although most frequently transverse. The system of fenestrated elastic lamellæ of the middle coat is sharply bounded externally, and is followed by the relatively thin adventitia. In consequence of the large quantity of elastic tissue contained both in the internal and middle coats, and the scanty muscular development of the latter, the distinction between the two inner tunics is less marked in the large arteries than in those of less size.

In a long section of the **inferior vena cava,** below the diaphragm, the thin intima is seen to have a structure somewhat similar to that of the arteries. A scanty elastic network, with mostly longitudinal threads, takes the place of the internal elastic lamina. This is followed by a thin layer of muscular fibres arranged transversely. Then the remainder of the wall consists of connective tissue, with an elastic network, and in which there run anastomosing bundles of longitudinally-disposed muscular fibres.

In sections of blood-vessels stained with picrocarmine the elastic tissue is stained yellow and the nuclei red. Double staining with logwood and eosin gives good results. The elastic tissue is red, the nuclei blue.

5. The endothelium of the larger vessels may be shown by laying open the vessel, and placing it in one-half per cent. nitrate of silver solution, having previously washed any blood away with distilled water. After a few minutes the vessel may be removed from the silver, washed, and exposed to the light in water until the inner surface assumes a brown colour. Portions of the in-

ternal coat are then stripped off with forceps, and mounted in glycerine, with the inner surface uppermost.

If a large vessel be acted on with nitrate of silver after the endothelium has been removed, there will be brought out in the internal coat a beautiful system of branching and anastomosing figures, appearing clear on a dark ground. These are spaces, and are occupied by flat, branching cells, now invisible, but which are seen as linear or spindle-shaped bodies in vertical sections through the coats of the vessel. In order to see the appearance described, it is essential that the endothelium be removed, which can be done by brushing the inner surface of the vessel with a camel's hair pencil. Here, as in the case of tendon and cartilage, silver stains the intercellular substance, but leaves the cells uncoloured.

Directions for injecting the vessels with coloured masses will be given in the Appendix.

CHAPTER IX.

LYMPHATICS.

Besides the blood-vessels, there exists another system of tubes in the body, namely, the lymphatics. These do not form a closed circuit, like the blood-vessels, but arise in the tissues at all parts of the body, and converge to two great trunks, which pour their contents into the subclavian veins, one on each side. They constitute a great drainage system, by which waste or superfluous matters are removed from the tissues, and conveyed back into the blood.

The larger lymphatic vessels resemble in structure the arteries, but their walls are much thinner, so that they collapse like veins, which vessels they further resemble in possessing valves. The endothelium is spindle-shaped, with sinuous outlines. The intima consists of connective tissue, with a small quantity of elastic tissue. An elastic network forms an imperfect internal elastic lamina, outside which is a layer of smooth muscular fibres, arranged transversely, or somewhat obliquely. Most externally is connective tissue, traversed by an elastic network.

Valves occur very numerously in the lymphatics. Their structure is similar to that of the venous valves, and above each pair of valves the lymphatic is dilated, so that the vessel, when distended, has not a cylindrical, but a beaded appearance.

In the **smaller lymphatics**, which usually form a network, or plexus, the muscular tissue is scanty, or absent. They possess an endothelium, whose cells have sinuous outlines, a connective tissue coat, and numerous valves. These vessels pass gradually into the **lymph capillaries**, whose wall is formed of a layer of irregularly-shaped, very sinuous endothelial cells only, and which have no valves. They have very irregular contours, and communicate at their peripheral extremity with a system of anastomosing spaces, which are called the **juice canals** (Saftkanälchen), or **lymph canalicular system**, and which are the origins of the lymphatic system of vessels.

These spaces in the firmer tissues, as tendons, fasciæ, cornea, bone, &c., have generally the shape of the connective-tissue-cells of these parts, and are, in fact, the spaces in the cementing material in which the connective-tissue-cells lie. These connective-tissue-cells may be looked on as an incomplete endothelial lining of the lymphatic spaces, and the endothelial lining of the lymph capillaries can frequently be seen to be continuous with the connective-tissue-cells lying in the juice canals (Klein). We have already seen these juice canals as the clear branching spaces manifested by nitrate of silver in tendon, and as the lacunæ and canaliculi of bone. It is affirmed that in cartilage a similar system exists, the spaces which contain the cells communicating one with another by fine channels. Other examples will subsequently be seen. In the loose formless connective tissue the spaces between the bundles of fibrous tissue are the origins of the lymphatics; while another origin is to be found in the serous cavities, whose communication, by stomata, with the lymphatics has been already noticed.

The lymph capillaries often expand, so as to form **split-like spaces, or lacunæ**, which surround certain parts, as the alveoli of acinose glands and some lymphatic follicles. In many places the blood-vessels lie in the interior of lymphatic channels, the so-called **perivascular lymphatics**. In these cases the outer surface of the blood-vessel is covered by endothelium, as well as the opposed surface of the lymphatic. These perivascular lymphatics may be looked on as split-like enlargements of the lymph capillaries, and, as in the other cases mentioned, are commonly traversed by connective tissue bands and blood-vessels, whose surface is always covered by endothelium.

In the course of the lymphatic vessels from the periphery towards the centre there are set numerous organs, which are called **lymphatic glands**, or lymphatic ganglia. Simpler bodies of the same nature exist in large numbers in connexion with the peripheral parts of the lymphatic system. It must be carefully borne in mind, that these organs, although they are called glands, have no ducts, or epithelial elements, and differ altogether from the true secreting glands; and also that they do not secrete the lymph, which is a transudation from the blood through the walls of the capillaries. The function of the lymphatic glands is only imperfectly known, but it is probable that it is intimately connected with the production of the cells of the lymph and of the blood.

Each lymphatic gland is somewhat kidney-shaped, more or less flattened and elongated, convex along one edge, and having a depression, or hilum, at the other.

It is surrounded by a **capsule**, formed of fibrous and elastic tissue, and containing in many animals a considerable quantity of smooth muscle. From the inner surface of this capsule there pass into the substance of the gland a number of processes, or **trabeculæ**, which circumscribe spaces. These spaces at the periphery of the gland are round; but in the deeper parts they are tubular, and run an exceedingly tortuous course, converging towards the hilum, where the trabeculæ from the capsule unite with a mass of connective tissue, which, accompanying the blood-vessels, penetrates the organ at this part. It must not be supposed that the spaces bounded by the trabeculæ are completely closed: they communicate freely with one another, so much so that the trabeculæ form much more a network of cords than a system of membranous walls.

The space between the fibrous septa is occupied by a peculiar tissue, called **adenoid tissue**, which has not yet been described. It belongs to the class of connective tissues, and consists of two parts—an exceedingly fine network of fibres, and a number of small round cells lying free in the meshes of the network. These so-called **lymphoid cells** resemble the white corpuscles of the blood, but they are smaller, and there is a less quantity of protoplasm surrounding the nuclei, of which each cell possesses only one. When the lymphoid cells are removed the **network** can be plainly seen. It consists of fine threads, with swellings at the points where they join, to form the reticulum. At these nodal points there are very commonly situated round or oval nuclei, and the appearance resembles very closely those which would be offered by a system of stellate cells anastomosing by their processes, and so forming a network. This conception of the nature of the tissue was formerly held as the true one; but it has been found that by mechanical means, such as brushing fine sections with a camel's-hair pencil, all the nuclei can be removed, while the network remains as before. Hence it is clear that the nuclei must lie, not in the interior of the swollen parts of the reticulum, but on the surface; and it is now known that the network of adenoid tissue is formed of fibres, which are covered by **flat endothelial cells**. The nuclei of these cells are those of which mention has been made as occurring at the nodal points of the reticulum, while between the fibres no cells or nuclei exist.

This adenoid tissue occurs in the lymphatic glands in two forms, which, however, differ only in degree. In one the threads of the network are comparatively coarse, and the meshes open; in the other the reticulum is extremely fine, and the meshes narrow. The boundaries between the coarse and the fine tissue are always sharply marked. The finer tissue lies always in the central portion of the spaces, which are bounded by the trabeculæ. At the periphery of the gland it forms rounded masses, which appear to the naked eye as little white granules, and are called the **cortical follicles**. In the deeper parts it forms cylindrical cords—the so-called **medullary cords**. The medullary cords and follicles are always separated from the capsule and the trabeculæ by an interval, which is occupied by the looser and coarser reticulum. Consequently, in a section near the surface rounded masses of dense tissue are seen, circumscribed by a clear space, across which run the threads of the coarser network, by which the dense central mass is attached to the capsule and trabeculæ. In the deeper parts, if the system of trabeculæ and cords is cut in length, we see two trabeculæ, appearing as fibrous bands; the mid-space between them is occupied by a cord of dense tissue, which is attached to the trabeculum on each side by the looser network. If the system is cut across, the medullary cord appears as a rounded portion of dense tissue, circumscribed by coarser tissue, which joins it to the surrounding trabeculæ. It is, of course, understood that the meshes of both coarse and fine tissues are occupied by lymphoid cells.

The **afferent lymphatic vessels** enter the gland at its convex side. They perforate the capsule, and open into the looser tissue about the follicles; from this the lymph passes towards the hilum, trickling always through the superficial looser tissue, and around the denser tissue, into whose meshes it does not enter. The looser tissue through which the lymph percolates is called the **superficial lymph path.** At the hilum the lymph is again taken up by the **efferent lymphatic vessels**, which enter the gland surrounded by a mass of connective tissue, and communicate with the terminations of the lymph paths. It will be seen that in its passage through the gland the lymph is not contained in proper vessels, but the superficial lymph path may be looked on as a system of lymphatic splits, such as have been described above. The trabeculæ and the surface of the dense adenoid tissue are covered by endothelium, which is continuous with that covering the threads of the coarser reticulum of the lymph paths, and with that of the afferent and efferent lymphatic vessels.

The lymphatic glands are freely supplied with blood. The **arteries** enter at the hilum, and the larger branches run in the trabeculæ. The smaller branches pass across the looser tissue, and the **capillary network** is formed always in the denser tissue of the follicles and medullary cords. The **veins** leave the gland at the hilum.

The **nerves** enter with the vessels, whose muscular coat they supply, as well as the muscular tissue of the capsule and trabeculæ.

It is probable that the lymph corpuscles are formed in the neighbourhood of the capillaries in the denser tissue of the gland, and that they then make their way into the lymph paths, from which they are removed by the lymph current. The meshes of the denser tissue communicate with those of the coarser network through the imperfect boundary of the former, so that there is an easy passage for the lymph corpuscles from one to the other.

In the **peripheral lymph follicles,** which are so common in the digestive and respiratory organs, the arrangement of parts in the lymphatic glands is repeated in a simpler form. Each follicle consists of a rounded mass of dense adenoid tissue, abundantly supplied with blood-vessels. The lymphatic vessels, however, do not penetrate the follicle, but lie external to it, forming about it either a close network or a split-like space. Each of these follicles resembles, consequently, one of the cortical follicles of a lymphatic gland.

The lymph, as collected from the lymphatic vessels, is a clear or slightly opalescent fluid, which coagulates after being withdrawn. It contains, in a transparent plasma, numerous corpuscles, resembling the white corpuscles of the blood. They vary a good deal in size, have usually a single nucleus, and present lively amœboid movements. A few red corpuscles are commonly found in the lymph, but they are probably an accidental ingredient.

THE THYMUS GLAND is a fœtal organ, which begins to waste at the second year, and disappears before adult age. It is covered by a fibrous capsule, which sends septa into the gland, dividing it into larger portions (lobes), and these again into smaller segments (lobules). Each lobule consists of a number of follicles or acini, which are separated at the periphery, but run together in the central parts of the lobule.

Each follicle is composed of adenoid tissue, but a sharp distinction into a coarser and finer reticulum, such as is found in the lymphatic glands, does not exist, although the tissue is somewhat denser at the periphery of each follicle than near the centre. The meshes of the network are occupied by lymphoid cells, and large endothelial-like cells are also to be found in places. Peculiar concentrically-laminated bodies occur in the follicles, and are called, after their discoverer, Hassall's corpuscles. Their real import is still a matter of doubt. The larger blood-vessels run in the centre of the lobules, and send numerous branches into the follicles, where they form an abundant capillary plexus. Of the lymphatics little is certainly known. According to Klein, lymph sinuses occur at the periphery of the follicles. The nerves are chiefly for the supply of the blood-vessels.

1. To inject lymphatic vessels it is necessary to employ the **method of puncture,** since if injection were forced into a vessel capable of holding a cannula, the valves would prevent its flowing backwards into the finer branches. A hypodermic syringe is filled with a saturated watery solution of soluble Prussian blue. The nozzle is thrust into the tissue to be injected, and gentle

pressure made on the piston. If the fluid accumulates about the point of the nozzle, the instrument must be withdrawn, and a fresh puncture made at some other place. After a few trials, it will be found that the injection runs off from the point of the syringe in fine lines, forming a network. These are the lymphatics, considerable tracts of which may thus be injected. The tissue is hardened in alcohol, and examined by sections or otherwise.

Lymphatics may also be injected through the serous cavities. If Prussian blue be injected into the abdominal cavity of a living rabbit, it will be found, after a few hours, that the lymphatics of the diaphragm are full of the blue fluid (Klein).

The same result may be got by killing a rabbit, and cutting it in two just below the diaphragm. The anterior half is suspended with the head downwards, a cannula placed in the trachea, some Prussian blue is poured on the abdominal surface of the diaphragm, and artificial respiration maintained for a few minutes. It will then be seen, on washing the diaphragm with a stream of water, that the lymphatics are full of the pigment, which also fills the lymphatics of the mediastina. Portions of the central tendon may be removed and placed in alcohol, and subsequently mounted in Canada balsam. With a lens or very low power of the microscope the network of lymphatics can be followed. The position of the valves will be apparent, and the dilatation of the tube above each valve will be distinct.

In these experiments the respiratory movements of the diaphragm distend and compress alternately the lymphatics on the lower and upper surface of the membrane. In this way the fluid is drawn in through the widely-opened stomata, and then propelled forwards towards the mediastina.

2. A rabbit is killed by hæmorrhage from the vessels of the neck. The thorax is carefully opened. A double ligature is placed around the vena cava just above the diaphragm, and the vessel divided between. The heart and lungs are removed. The diaphragm is made to bulge by pressing gently on the abdomen. With a large soft camel's hair pencil, moistened in distilled water, the surface of the central tendon is gently brushed, so as to remove the pleural endothelium. Then nitrate of silver, one-half per cent., is poured on the diaphragm, and allowed to remain a few minutes, after which it is washed away by a current of distilled water, and the central tendon is carefully removed, placed in alcohol, and exposed to the light. When the reduction has taken place, there will be seen, even with the naked eye, a network of clear lines on the brown ground. Portions of the tendon may be mounted in glycerine or in Canada balsam. The lymphatics will be seen as clear lines. Their general arrangement, the shape of their endothelium, and their valves will be evident. The very irregular lymph-capillaries, which may be known by their very sinuous endothelium and the absence of valves, will be seen to open out

at the end and sides into a system of clear branching spaces: these are the juice canals.

3. If the omentum of a recently-killed rabbit be spread out, and its surface carefully brushed, to remove the endothelium, and hen stained in silver and mounted in glycerine, very beautiful views of the blood-vessels and lymphatics may be got (Klein). The different shape of the endothelium in the arteries, veins, and lymphatics, the valves in the lymphatics, and the muscular coat of the arteries may be seen. In successful cases the juice canals also appear as irregular branching and anastomosing figures. The preparation is, however, difficult to make.

4. The silver preparations of tendon may be referred to, to see the juice canals. These are the clear branching figures brought out by treating a tendon with nitrate of silver after the removal of the surface endothelium. It was seen that these were spaces in the cementing material, that they were situated on the outside of the tendon-bundles, and that they contained the flat connective-tissue cells.

5. Preparations of the cornea of the frog stained, one in chloride of gold, and another in nitrate of silver, should be examined. In the gold preparation it will be seen that the connective-tissue cells are flat, branching plates, anastomosing by their processes, and the nerve trunks appear as cords ramifying over the field. The nerves and the connective-tissue cells are stained of a purple colour; the intervening tissue is unstained. In the silver preparation the intercellular substance is stained, and the spaces in which the cells lie—in other words, the juice canals—appear as clear, stellate and anastomosing figures. The channels in which the nerve trunks run are clear, and show an endothelium. Into these channels some of the juice canals open. Except the nerve sheaths, there are no lymphatic vessels in the cornea. The structure of the cornea will be subsequently described in detail.

6. In preparations of the mesentery of a frog, whose blood-vessels have been injected with nitrate of silver, the endothelium of the perivascular lymphatics is often stained by transudation of the silver through the walls of the arteries and veins, and these vessels may be seen to be surrounded partially or completely by lymphatic channels.

7. Lymphatic glands may be hardened in alcohol alone, or in Müller's fluid and subsequently alcohol. The hardening must not be too great, as it then becomes difficult to remove the lymph corpuscles, and the tissue becomes brittle.

Sections are best made with the microtome, and should extend from the hilum radially towards the convex surface. The sections should be stained in logwood or picrocarmine, and then placed in a test tube half filled with water, and shaken for some time, so as partially to remove the lymph corpuscles, which would otherwise conceal the reticulum. The sections are then placed in a saucer of water, floated on to a slide, and mounted in glycerine. The tissue is exceedingly delicate, and must be handled with great care.

The fibrous capsule and the trabeculæ are seen. The lymph corpuscles are completely removed from the coarser tissue, but still lie in most parts of the denser tissue. The large follicles at the surface of the gland are probably much broken, and to see them it is best to mount a section without shaking. The medullary portion, however, is well seen, and the appearances should be readily understood from the description given above.

8. If a lymphatic gland be injected with Prussian blue by puncture, the injection will be seen in the superficial lymph paths. Sections of glands so injected, and then stained in picrocarmine, form beautiful and instructive objects. The medullary cords and follicles are bright red, from the number of lymph corpuscles they contain. These red parts are seen to be always separated from a trabeculum by a blue border, which is the injection contained in the superficial lymph path.

9. Sections of lymphatic glands whose blood-vessels have been injected should be examined. It will be seen that the capillary network is never in the lymph paths, but always in the denser adenoid tissue. It will also be seen how the reticulum of this tissue is attached to the outer surface of the walls of the blood-vessels, forming for them a kind of adventitious coat.

10. The thymus gland of a newly-born child should be hardened in Müller's fluid and alcohol. Sections stained in logwood, or, still better, first in logwood and then in eosin, and mounted in glycerine.

The follicles are seen; separated at the outer part of the lobule, but confluent in its deeper part. Each follicle is composed of adenoid tissue, whose lymph corpuscles are more numerous near the periphery than at the centre. The network of capillary blood-vessels, often containing blood, and the concentrically-laminated corpuscles of Hassall, should be noticed.

CHAPTER X.

THE SPLEEN.

THE spleen is covered by peritonæum, which consists here, as elsewhere, of a layer of endothelium resting on a fibro-elastic membrane. Under this is the proper **capsule,** composed of firm connective tissue, and which contains, particularly in some of the lower animals, a considerable number of smooth muscular fibres, arranged in bundles, which cross and anastomose with one another.

At the inner or concave surface of the spleen is the hilum, or place where the large blood-vessels enter and leave the organ. These vessels are surrounded by connective tissue, which enters with them at the hilum, and accompanies them for some distance into the interior of the spleen. With this connective tissue the capsule becomes continuous at the hilum.

From all the inner surface of the capsule processes pass into the interior of the spleen. These are called the **trabeculæ.** They are composed, like the capsule itself, of connective tissue and smooth muscular fibres. They are cord-like or flattened bands, which branch freely, and, anastomosing with one another, form a framework for the support of the more delicate tissue which lies between them. Some of them reach and become continuous with the connective tissue which enters at the hilum ; others break up in the interior of the spleen into fine threads, which are lost in the pulp. The large **blood-vessels** enter at the hilum, surrounded by connective tissue. At first the arteries and veins run together, but when, by repeated branching, the arteries have been reduced to a certain size, they part company with the veins. The **arteries** and their branches run a radiating course towards the surface of the spleen, and they are peculiar in not anastomosing with one another, but each constitutes what is called a terminal artery. If any artery be taken, it will be found that it and its branches form a conical figure, whose base is towards the capsule, and whose apex is turned towards the hilum; and the vessels composing this cone communicate neither with each other nor with those of neighbouring cones. This distribution of the arteries explains the appearances which are so commonly seen in cases where embolism of one of the splenic arteries has occurred.

For some distance after they leave the veins the arteries continue to be surrounded by a sheath of fibrous connective tissue, but this gradually changes its character, and becomes converted into adenoid tissue, quite similar to that which occurs in the lymphatic glands. The adenoid sheath accompanies the arteries to their termination. It is narrow in places, and in others it bulges out so as to form large rounded or irregularly-shaped masses, which have long been known as the **Malpighian corpuscles** of the spleen. These can be seen on sections of the fresh spleen as round spots, which contrast by their whitish colour with the dark red spleen-pulp. The artery generally lies, not in the centre, but near one side of the Malpighian corpuscle. The adenoid tissue of the arterial sheaths, with their swellings forming the Malpighian corpuscles, is supplied with an abundant network of capillary blood-vessels. The arteries either terminate in this network, or some of them break up into a number of minute twigs, which open into the spleen-pulp.

Filling up the space between the trabeculæ and the Malpighian corpuscles, and attached to both, is the third element of the spleen, or **the pulp.** This is a fine reticulum, with nuclei at the nodal points, and formed apparently of branching and anastomosing cells, many of which are much flattened. In the interstices of this network are contained great numbers of red blood corpuscles, white corpuscles, and larger cells, resembling those found in the red marrow of the bones, and which sometimes contain red blood corpuscles in their interior. Into the meshes of the pulp the capillaries and terminal arterial branches open, so that here the blood suffers an actual extravasation, and does not run in closed vessels.

The veins enter the spleen in company with the arteries, and are enclosed with them in a fibrous sheath. They at first have a muscular coat, with chiefly longitudinal fibres, but as they diminish in size they lose this. The smaller veins run in the pulp, and branch freely. Their wall is formed of transversely-placed elastic fibres, on the inner aspect of which is a layer of spindle-shaped endothelial cells, whose nuclei frequently lie in little projections from the inner surface of the cells. This wall gradually becomes imperfect by separation of its elements, and the capillary veins, like the arteries, open into the pulp-spaces. The blood then, in its passage from the arteries to the veins, has to trickle through the pulp, just as in the lymphatic glands the lymph does through the adenoid tissue of the lymph paths.

No such extravasation of blood occurs in the Malpighian corpuscles. In them the circulation is carried on through closed capillary vessels. There is no perfect septum between the pulp and the Malpighian corpuscles, but the tissue of the latter is condensed at its outer part.

The **lymphatics** of the spleen run partly in the capsule and trabeculæ, in which structures they form a network (superficial lymphatics). There are also lymphatics which accompany the larger blood-vessels, and whose origin seems to be in relation with the Malpighian corpuscles (deep or perivascular lymphatics). These two systems communicate with one another at the hilum.

The **nerves** of the spleen are numerous, and consist mostly of non-medullated fibres. They accompany the arteries, whose muscular coat, as the muscular tissue of the capsule and trabeculæ, they supply. Fibres have been traced to peculiar elipsoidal structures, consisting of connective tissue arranged in lamellæ around vessels which are usually looked on as capillaries, but which Klein considers to be arteries.

1. Scrapings from the cut surface of a fresh spleen examined in 0.5 per cent. salt solution show numerous red blood corpuscles, cells resembling white blood corpuscles, and other colourless cells larger than these. In some cases these latter cells contain in their interior red corpuscles, which seem to be undergoing destruction. These blood corpuscles-holding cells are very common in many morbid conditions, specially typhoid fever, in which disease the spleen pulp undergoes a great hypertrophy. There are also to be seen numbers of the endothelial cells of the veins. These, viewed from their edge, often appear as crescentic bodies, each with a projection, which contains the nucleus on its concave side.

2. Section of a spleen which has been hardened in Müller's fluid and alcohol, stained in logwood, mounted in Canada balsam. With a low power the three elements are readily recognised. The trabeculæ appear as fibrous cords, continuous at their outer ends with the capsule. In these the spindle-shaped nuclei of smooth muscular fibres are seen. The Malpighian corpuscles appear as round or irregularly-shaped patches, stained of a bright-blue colour, owing to the number of lymph corpuscles, whose nuclei appear as blue points. In the interior of each Malpighian corpuscle an artery is seen. Between the Malpighian corpuscles and the trabeculæ is the pulp, of a brownish colour, in consequence of the blood which is contained in its meshes. With a high power the blood corpuscles, and, if the section be very thin, the details of the structure of the reticulum of the pulp, can be seen. The smaller veins have usually collapsed, and are visible only in very good preparations.

3. Section of a spleen whose blood-vessels have been injected from the arteries with Prussian blue; cochineal; balsam. The network of capillary vessels in the Malpighian corpuscles is seen. In the pulp there are no capillaries, but the injection is everywhere extravasated, and fills all the interstices of the tissue. It can be well seen how the veins commence by open mouths in the pulp.

CHAPTER XI.

DIGESTIVE ORGANS.

I.—*Mouth and Pharynx.*

THE mucous membrane of the mouth is covered by a compound scaly **epithelium**, having the characters already described.

This rests on a basement membrane, which is believed to consist of flattened cells. Underneath this is the **mucous membrane,** composed of bundles of fibrous tissue, with flat connective-tissue cells, the bundles being closely interlaced, so as to form a firm membrane. In some parts of the mouth the mucous membrane is intimately attached to the subjacent parts, as to the muscular tissue on the dorsum of the tongue, and to the periosteum on the gums and hard palate; but at other places, as on the floor of the mouth, it has beneath it a considerable quantity of areolar **submucous tissue**, containing fat.

The surface of the mucous membrane is not smooth, but is covered with a number of minute elevations, called **papillæ.** These are generally conical in shape, and project into the epithelium, but do not cause an elevation on its free surface, so that the layer of epithelium is much thinner over the summits of, than in the valleys between, the papillæ. The papillæ consist of the fibrous tissue of the mucous membrane, and contain loops of capillary blood-vessels and nerves, which on the lips, and perhaps elsewhere, terminate in peculiar organs, known as tact corpuscles.

On the **dorsum of the tongue** there exist papillæ of very large size, which, because they bear on their surface secondary papillæ, are distinguished from those already described by the name of **compound papillæ**. Of these there are on the human tongue three kinds: 1. The most numerous are called **filiform** and consist each of a small conical or cylindrical elevation of the mucous membrane, on whose upper surface are numerous smaller elevations. The entire papilla is far too large to lie altogether in the thickness of the epithelium, but carries upon its sides, above the general surface, a covering of epithelium, consisting of all its layers. On the upper surface of the papillæ the epithelium becomes enormously thickened, particularly the superficial layers, whose flattened horny scales are felted together, so as to form long, hair-like processes, several of which may arise from a single papilla. It is these hair-like processes which form the fur on the tongue. When the tongue is foul there are generally found adhering to the epithelium, masses of fungi, bacteria, and other putrefactive organisms.

2. In much smaller number than the filiform papillæ, but still very generally distributed, are the **fungiform papillæ**. Each of these is a rounded elevation of mucous membrane, somewhat constricted at its base; its surface is everywhere covered over by secondary papillæ, embedded in the epithelium, which is thin, and, in consequence of its thinness, allows the blood contained in the capillary loops to show. Hence these fungiform papillæ appear on the tongue as minute red points, and when, as in some dyspeptic conditions and after scarlatina, there is a desquamation of epithelium, it is these papillæ which give to the tongue its strawberry appearance. The fungiform papillæ contain numerous blood-vessels, which form loops in the secondary papillæ, and also nerves, some of which terminate in peculiar epithelial structures, known as taste-buds, whose description will be given with that of the organs of special sense.

3. Near the root of the tongue, forming a single

line, in the shape of a V, with the apex turned backwards, are eight or more large papillæ, each of which is of cylindrical or slightly fungiform shape. These are the **circumvallate papillæ.** They have the peculiarity of being each surrounded by a circular depression, or fossa. It is from the bottom of this fossa the papilla properly springs, and its summit extends very little above the general level of the mucous membrane. The circumvallate papillæ have secondary papillæ on their flattened upper surface, but none on their sides. They contain blood-vessels and numerous medullated nerves, and taste-buds are abundant on the sides of the papillæ and on the opposed side of the fossæ.

Besides the three forms of papillæ which have been noticed, transitional forms between filiform and fungiform have been described as **conical papillæ.** They have no taste-buds. On the side of the tongue, far back, there is a part where the mucous membrane is thrown into a number of transverse folds. This is the **papilla foliata,** an organ which is much more highly developed in the rabbit than in man. The epithelium here contains numerous taste-buds.

The papillæ on the tongues of the lower animals often differ considerably from those occurring in man, particularly the filiform papillæ, whose epithelium is in many animals condensed to form hard horny spines.

The line of the circumvallate papillæ separates the dorsum of the tongue into two well-defined regions. All in front of this line is level, but velvety, in consequence of the numerous papillæ which cover the surface ; but posteriorly the surface is uneven, being occupied by a number of rounded elevations, separated by depressions. Here there are only simple papillæ, which lie altogether in the thickness of the epithelium, and consequently the velvety appearance is absent. On examining the elevations, many of them are seen to present at their summit a minute hole, leading into a flask-shaped cavity, which is lined throughout by compound scaly epithelium. The mucous membrane forming the wall of these cavities consists of adenoid tissue, which is sometimes uniformly distributed, but often consists of little rounded masses (the so-called follicles), separated by tissue which, although containing numerous lymph corpuscles, is not a reticulum, but fibrous. Here, and wherever epithelium rests immediately on adenoid tissue, the limits between the two tissues are badly defined, in consequence of the deeper layers of the epithelium being infiltrated with lymph corpuscles (Klein). These depressions in the mucous membrane, with their walls of adenoid tissue, are called the **follicular glands** of the tongue.

In front of the circumvallate papillæ there is no adenoid tissue. On the under surface of the tongue there exist only small simple papillæ, which are embedded in the thin epithelium.

The **proper substance of the tongue** is composed of striped muscular tissue, whose fibres run, some from behind forwards, others from below upwards, others from side to side. The fibres which pass upwards branch, and are inserted by short tendons into the deep surface of the mucous membrane. Those which pass inwards are inserted into a fibrous median septum, which divides the tongue into two lateral halves. Between the muscular fibres is a considerable quantity of fat. Numerous large arteries, veins, and nerves, and their branches, occur in the tongue.

Numerous glands open into the mouth. These, although differing much in size and details of structure, are all formed on the same type, and are all called acinose glands.

An acinose gland consists of a duct, which branches very freely, and whose terminal divisions open into dilated cavities, generally of an elongated tubular form. These are called acini, and are lined by the epithelium, which produces the secretion.

According to the character of the secretion, the glands of the mouth are divided into two classes. In one class the secretion is viscid, and contains mucin. These are **mucous glands.** In the other the secretion is limpid and watery, and contains a good deal of proteid. These are **the serous, or albuminous glands**, or true salivary glands.

The glands are also divided into large and small. The large are the parotid, submaxillary, sublingual, and Nuhn's glands, which lie under the point of the tongue. The small glands are scattered over the interior of the mouth, being peculiarly numerous on the insides of the lips, interior of the cheeks, soft palate, and back part of dorsum of tongue, under and behind the circumvallate papillæ.

Of the larger glands, the parotid is serous in all animals; the sublingual and Nuhn's glands are always mucous; while the submaxillary is sometimes mucous, as in the dog, cat, &c.; sometimes serous, as in the rabbit; sometimes partly one and partly the other, as in man.

Of the smaller glands, all are mucous, except a group on the dorsum of the tongue, extending across under the circumvallate papillæ, and whose ducts for the most part open into the fossæ surrounding these.

The larger salivary glands can be seen to be divided into a number of lobes, by the passage into them of loose connective tissue continuous with that which forms their capsule. The lobes are again similarly divided into smaller divisions (lobules), which give to the gland its granular appearance.

The principal **duct** opens into the mouth, and is composed of connective tissue, with more or less smooth muscular fibres, and is lined by a simple columnar epithelium. It divides freely on its arrival at the gland, the branches going to the different subdivisions of the latter. On its entrance into the lobules it changes its character. Its wall becomes thinner, and no longer contains muscular fibres. It is lined by a single layer of high, columnar, epithelial cells, each containing about its middle a round nucleus, and presenting in its outer part a very distinct radial striation. In this stage the duct is known as a **salivary tube.** The salivary tube narrows rather suddenly, and passes into one, sometimes several, very fine canals, formed of a basement membrane and a single layer of flat or low cubical epithelium. The point of narrowing is called the neck, and here the cells are small, cubical, and closely placed (Klein). The minute terminal canals or terminal ducts open into the **acini.** These are tubular spaces of considerable diameter; but, in consequence of the depth of the epithelium, the lumen, or central cavity, is small. The wall is formed of a basement membrane, strengthened by stellate cells, which lie on its inner side, immediately beneath the epithelium. **The epithelium**, of the kind known as glandular, differs according as the gland is mucous or serous. In the latter case the cells are polygonal or pyramidal, of uniform finely-granular structure, and with round nuclei. In the mucous glands the acini are larger, the epithelial cells clear and transparent, and the nucleus is flattened, and lies close to the basement membrane. At certain parts of the acini, external to the mucous cells, are groups of granular cells, with round nuclei, sometimes forming crescentic-shaped figures, as in the submaxillary and orbital glands of the dog; at others, as in the submaxillary glands of the cat, almost completely surrounding the acinus, which thus has two layers of cells—an inner mucous and outer granular. These **semilunes, or crescents of Giannuzzi,** vary in distinctness in different glands, the variations depending largely on the condition of the gland as to activity or rest at the time of the death of the animal to which it belonged.

The salivary glands are abundantly supplied with **blood-vessels,** the capillaries forming a plexus around the acini. The **lymphatics** begin as splits or fissures around the acini, and pass into larger vessels, which lie in the connective tissue. The **nerves** are numerous, but their terminations are unknown, as is their relation to the groups of **ganglion cells,** which are commonly found embedded in the glands.

The **smaller salivary glands** are constructed on the same plan as the larger. The **ducts** consist only of a basement membrane and a columnar epithelium. They do not form salivary tubes, but become progressively smaller as they branch, and finally open into the **acini**. These are for the most part of a mucous character. They are comparatively large, and their epithelium is clear and transparent, with flattened nuclei situated near the basement membrane. Crescents of granular cells, such as occur in the larger mucous glands, are, as a rule, absent.

Situated under the surface of the dorsum of the tongue, in the region of the circumvallate papillæ, are glands, whose acini are small, whose epithelium is granular, with round nuclei. These are serous glands. Their ducts open into the fossæ about the circumvallate papillæ. The part of the dorsum of the tongue anterior to this has no glands, but posteriorly, as far as the epiglottis, a continuous layer of mucous glands exists. They lie embedded between the muscular fibres of the tongue, and their ducts open either into the crypts of the follicular glands, or directly on the surface.

The small glands in the lips, palate, and cheek are all mucous, and are situated either in the submucous tissue, or, when this is wanting, among the muscular fibres.

The tonsils are organs similar to the follicular glands of the tongue. Each tonsil consists of a mass of adenoid tissue, in which the follicular structure can often be very distinctly seen. Into this adenoid tissue dip involutions of the epithelium, forming a number of blind pouches, which open on the surface, and are lined by compound scaly epithelium. These are the so-called crypts of the tonsil. In some animals there is only one crypt, and then, except for its size, the tonsil precisely resembles a follicular gland of the tongue; but in man the crypts are numerous and branched. The tonsil is bounded by a dense fibrous capsule. Behind the tonsil lie numerous mucous glands, whose ducts pierce the adenoid tissue, and open into the crypts.

At the upper and back part of the pharynx is a structure similar to the tonsil, and known as the **pharyngeal tonsil.**

All these masses of adenoid tissue contain an abundant plexus of capillary blood-vessels.

The follicles are surrounded by a plexus of lymphatic vessels, or by a sinus-like fissure, lined by sinuous endothelium.

The upper part of the pharynx is lined by columnar ciliated epithelium, and belongs to the respiratory passages. The lower part is lined by compound scaly epithelium, and has numerous simple papillæ. In both parts mucous glands are abundant.

Teeth:

That part of the tooth which projects above the surface is called the **crown**. The slightly constricted part which is embraced by the gum is the **neck,** and the part which is embedded in the alveolus is the **fang.** A tooth is a hollow organ. The cavity in its interior is called the **pulp cavity.** It has fine openings, one at the point of each fang, by which the blood-vessels and nerves pass into the interior of the tooth.

The bulk of the tooth is formed of **dentine.** This is a tissue, consisting, like bone, of fine fibres held together by a calcified cementing-substance. It bounds the pulp cavity, and from this there pass into the dentine a number of fine tubes, which run a generally radiating course towards the surface of the dentine, branching freely, and anastomosing by their branches. The walls of these dentine-tubes are formed of a substance, probably of a horny nature, which differs from the remainder of the dentine in possessing a greater power of resistance to the action of acids.

On the crown of the tooth the dentine is covered by **enamel.** This consists of a number of long hexagonal columns, resting by one end on the surface of the dentine, and by the other reaching the free surface of the tooth. These columns present slight varicosities, and are held together by a firm cementing-substance. They are arranged in groups, and the directions of the columns in neighbouring groups differ slightly. The surface of the enamel in young teeth is covered by a thin, homogeneous, calcified cuticle (enamel cuticle). Irregular cavities exist frequently between the deeper parts of the enamel-columns. These communicate with the dentine-tubes.

The enamel is thickest on the most projecting part of the tooth, and gradually thins off towards the neck, where it ceases.

On the fang of the tooth the dentine is covered by a layer of bone, the **cement** or **crusta petrosa.** It contains lacunæ of large size, and canaliculi which communicate with the dentine tubes. There are, as a rule, no Haversian canals in the crusta petrosa.

The crusta petrosa begins as a thin layer at the neck, meeting the enamel, and becomes thicker on the deeper parts of the fangs. Owing to this arrangement, the dentine is completely covered, but there is sometimes a narrow zone where this tissue is exposed between the enamel and the crusta petrosa.

The outer parts of the dentine are frequently imperfectly calcified, and in macerated teeth these parts present a number of irregularly-shaped cavities, into which the dentine tubes open, and which communicate with the lacunæ of the crusta and with the cavities which have been already described as occurring in the enamel. These imperfectly calcified portions of dentine are called the **interglobular spaces.** In the recent tooth they are occupied by uncalcified tissue, containing branched cells.

The cavity in the interior of the tooth is occupied by a soft tissue, called **the pulp.** This consists of a very soft, delicate connective tissue, containing numerous stellate and spindle-shaped cells, which anastomose by their processes, and form a reticulum. The cells which lie on the surface of the pulp where it touches the dentine are large and columnar, and arranged closely side by side, like an epithelium. They communicate with one another and with the other cells of the pulp by processes, and also send long processes into the dentine tubes. These cells are peculiarly well developed in growing teeth, and as it is from them that the dentine is formed, they are called **odontoblasts**. There is a close plexus of blood-vessels in the pulp and numerous nerves, from which fine twigs pass into some of the dentine-tubes (Boll).

The whole tooth is a modified and partially calcified papilla. The basement membrane runs between the enamel and the dentine. The enamel is epithelium ; the dentine, crusta, and pulp are connective tissue, and belong to the mucous membrane.

The tooth is held in its socket by a firm fibrous connective tissue, continuous with the membrane covering the tooth, or **peridontium.**

The **gum** consists of dense fibrous tissue. It has very large and regular papillæ, and is covered by a compound scaly epithelium.

1. The **tongue** of a cat is cut transversely into pieces, and hardened in one-sixth per cent. chromic acid for ten days, then in alcohol.

Transverse sections made in a plane perpendicular to the dorsum are stained in logwood, and mounted in Canada balsam.

The **muscular fibres** will be seen, some cut transversely, others running in the plane of the section. Of these latter, a number ascend vertically towards the dorsum, and are inserted into the mucous membrane. Between these are groups of punc- cut across. The peripheral position of the nuclei, and the fibres tated appearance of the section, due to the primitive cylinders, will be well seen in the fibres divided transversely. Other fibres, more deeply situated, run from side to side, and meet in the middle a fibrous septum or raphé. Between the muscular fibres is much **fat.** In the inferior parts of the section large **arteries, veins,** and **nerves** will be seen cut across, and many smaller vessels in other parts.

The **mucous membrane** of the dorsum is thick, and consists of fibrous tissue, with numerous small vessels. The **papillæ** are large, most of them pointed; the **epithelium** covering them thick and horny in its superficial layers, which do not stain. Between the papillæ the different layers of cells composing the epithelium can be well seen. On the under side of the tongue the surface is smooth and the epithelium thin, with simple papillæ.

2. Vertical sections through the dorsum of the back part of the human tongue (hardened as above) made parallel to the long axis of the organ, and extending to a depth of about half an inch below the surface. The section should go through the line of circumvallate papillæ. Stain in logwood, and mount in Canada balsam. In some of the sections **circumvallate papillæ** will be met, and the ducts of the serous glands opening into their fossæ will be seen. Anterior to them are the **filiform** and **fungiform papillæ,** while posteriorly the larger papillæ are absent, and the **adenoid tissue** of the follicular glands is seen just under the surface.

Embedded between the muscular tissue are the **secreting glands.** The acini, being cut in different directions, appear as round or oval figures, lying in groups, while the ducts are seen passing upwards and opening on the surface. The acini of the **serous glands** are small; the epithelial cells granular, with round nuclei. They lie under the circumvallate papillæ. More posteriorly are the **mucous glands,** with larger acini; clear, distinct epithelial cells, often staining a beautiful blue in logwood, and with compressed nuclei situated near the basement membrane.

Sections stained first in picrocarmine and then in iodine green are very pretty; the mucous glands and adenoid tissue taking a green colour, the other structures orange or red. They are, however, not more instructive than a simple staining, and the green colour seldom holds for very long.

3. Sections of a tongue whose blood-vessels have been injected. The close capillary plexus of the muscular tissue is beautifully seen; the loops of vessels passing into the papillæ, and the plexus surrounding the glands.

Mucous Gland:

4. Submaxillary gland of a dog or cat hardened in alcohol. Sections must be very thin, stained in logwood or picrocarmine, and are best mounted in glycerine or Farrant's solution, as many of the finer details are lost in Canada balsam preparations.

With a low power it is seen that the section is marked out by tracts of loose connective tissue into irregular-shaped divisions. These are the lobules. In the separating connective tissue are branches of the principal ducts and blood-vessels.

In the lobule the acini are seen as round, oval, or elongated objects, according to the direction in which they have been cut. Each is bounded by a distinct line, the basement membrane, on which are seated the large, clear, very distinct mucous epithelial cells. The nuclei of these cells appear flattened and situated close to the periphery. The cells do not stain in picrocarmine, but become a blue colour when stained in logwood. In places between the mucous cells and the basement membrane are seen crescentic figures formed of granular cells, with round nuclei and indistinct outlines. These cells stain a deep blue in logwood, and red in picrocarmine. In the cat the acini are smaller, and the crescents occupy a greater segment of their circumference than in the dog.

The intralobular ducts or salivary tubes will be seen cut in length or across. Their narrow lumen, high columnar epithelium, with round nuclei and radially striated protoplasm, make them very conspicuous objects. Their epithelium stains orange in picrocarmine. In fortunate sections the passage of salivary tubes into smaller ducts, and of these into acini, may be seen. In the submaxillary of the dog, the salivary tube passes into the acinus by a short portion, lined by cubical cells, with small, crowded nuclei (Klein). Between the acini and ducts is connective tissue, containing often numerous lymph corpuscles, and larger coarsely granular cells (plasma cells). The blood-vessels, except the larger ones, are not seen, unless the preparation is injected. Ganglia may be met. The nerve-cells, with large vesicular nuclei and endothelial sheaths, lie in groups generally in the interlobular tissue, near the large blood-vessels.

5. **Serous Gland.**—Submaxillary gland of a rabbit treated as the last object. The arrangement in lobules and the ducts are the same as in the mucous gland, but the acini are smaller. The epithelial cells are granular, and stain in picrocarmine. Their outlines are less distinct, and the nuclei are round. Besides these cells there are no others in the acini. The lumen of the acini is very small.

6. **Mixed Gland.**—Human submaxillary gland treated in the same way as 4 and 5. Most of the acini are of the serous type, but some are lined by mucous cells without crescents. In some acini there may be found granular and mucous cells side by side.

7. Small fragments of a fresh salivary gland, macerated one or two days in five per cent. neutral chromate of ammonia, may be torn up in the same fluid. The epithelial cells are seen separated or coherent in groups; also the stellate cells, which lie inside the basement membrane of the acini, and which appear to send processes between the epithelial cells. The manipulation is difficult.

8. The **tonsil** of a cat, hardened in one-sixth per cent. chromic acid and alcohol. Section through longest axis; stained in logwood, and mounted in Canada balsam. The organ is a long, narrow pouch, opening at one end into the mouth, and closed at the other. It is lined by compound scaly epithelium, and its wall is formed of adenoid tissue, in which the follicular structure is remarkably distinct. It will be observed that the periphery of each follicle stains more deeply than the central part. This is common to all lymphoid follicles, and is due to the greater accumulation of lymph corpuscles at the periphery. Between the follicles the lymph corpuscles are numerous, but they lie in fibrous tissue, not in a reticulum. The adenoid tissue is bounded externally by a strong fibrous capsule. Outside this will be seen groups of mucous glands and fragments of the muscles of the palate or pharynx.

9. Section through a human tonsil prepared in the same way as 8. The organ here is oval, covered on its free surface with scaly epithelium. This lines also numerous branched pouches, which extend into the tonsil. The whole is bounded externally by a strong capsule (which sometimes sends septa into the organ), and all between this and the surface is formed of adenoid tissue, in which the follicular structure is not so distinct as in the tonsil of the cat. External to the capsule, mucous glands will be found, and sections of the muscular tissue of the palate and pharynx.

Teeth, like bone, may be prepared in two ways:—They may be decalcified by immersion in dilute acid, and then cut with a razor; or, after being deprived of their soft parts by maceration in water, they may be ground down into thin plates, and mounted in hard Canada balsam.

10. The lower jaw of a kitten is dissected out from the mouth, cut into short pieces with a fine saw, and placed in the mixture of chromic and nitric acids, which must be frequently changed. When the tissue is softened it is washed for some time in water, and then placed in spirit. Sections at right angles to the length of the horizontal ramus, and passing through the axis of a tooth; stained in logwood; mounted in Canada balsam.

The enamel consists almost entirely of earthy matter; consequently it is altogether dissolved away. The general shape of the tooth and its relation to surrounding parts will be seen; also the dentine, with its tubes appearing as indistinct radiating lines; the pulp-cavity, with its contents; the layer of odontoblast, lying against the inner surface of the dentine; the firm fibrous tissue, which binds the tooth in its socket; the spongy bone of the lower jaw; the gum, with its thick epithelium and long papillæ; and the inferior dental canal, containing nerve trunks, arteries, and veins, all cut across. In some sections, between the inferior dental canal and the milk tooth the developing secondary tooth will be seen.

11. A dry tooth, from which the soft parts have been removed by maceration in water, preferably a tooth of simple form, such as a canine, is ground down, first on one side, then on the other, so as to get a thin lamella going through the middle of the tooth, parallel to the long axis. This lamella is mounted in hard Canada balsam, according to the method directed for bone. The grinding is long and laborious, unless it is done with a lathe. The general arrangement of the three hard tissues will be distinctly seen, even with the naked eye. With the microscope the fine lines separating the enamel columns will be seen. The enamel presents radiating bands, alternately light and dark. This is due to the varying direction of the groups of columns, some of which are cut in length, others more or less across. Besides these radial markings, there are others parallel to the surface, and which are known as the lines of Retzius. Their import is uncertain.

The dentine tubes appear as distinct black lines, because they are filled with air, by which the light is reflected from their under surface, and is thus prevented from reaching the eye. They give off very fine and numerous branches, and anastomose with one another. They can be seen to pass into the interglobular spaces, which now contain only air, and to communicate with the cavities in the deeper part of the enamel, and with the canaliculi of the cementum, which, with the lacunæ, also contain air. Where the interglobular spaces do not exist, the dentine tubes terminate by blind extremities, or by anastomosing in loops, or by direct anastomosis with the canaliculi of the crusta petrosa.

On the dentine, lines may be seen parallel to the surface. These are the lines of Schreger, and are due to bendings of the dentine tubes.

II.—*Digestive Tube.*

The proper digestive tube begins at the œsophagus, and from this point downwards it is formed of certain layers, or **coats,** which are from within outwards the following :—

1. Mucous coat (*tunica mucosa*)
    - Mucous coat proper (*tunica mucosa propria*).
    - Muscular layer of mucous membrane (*tunica muscularis mucosæ*).
2. Submucous coat (*tunica submucosa*).
3. Muscular coat (*tunica muscularis*),
    - Circular layer.
    - Longitudinal layer.
4. Serous coat (*tunica serosa*).

In the œsophagus and lower part of the rectum there is no serous coat, but its place is taken by a layer of connective tissue, called the adventitious coat (*tunica adventitia*).

It is common in speaking of these coats to call them by their Latin names, omitting tunica. Thus we speak of the mucosa, the muscularis mucosæ, the serosa, &c.

The **adventitious coat** is formed simply of connective tissue, and the **serosa** is a covering of the peritonæum, whose structure has been already described. No more consequently need be said of these layers.

The **muscular coat** consists everywhere of two layers, whose fibres cross each other at right angles. In the external layer the direction of the fibres is longitudinal, or in the direction of the tube, while in the internal layer the fibres are arranged transversely. The muscular tissue is of the unstriped variety everywhere, except in the upper part of the œsophagus. Here it is of the striped kind. Among different animals there is considerable difference as to the point in the œsophagus at which the two kinds of muscular tissue meet. In man, from the level of the fifth dorsal vertebra downwards unstriped tissue is found; for some distance above that the two kinds of muscular fibres are mixed.

The unstriped muscular tissue is divided into bundles by septa of connective tissue.

Internal to the muscular coat is the **submucous coat.** This consists of loose areolar tissue, and in places contains fat. It allows the mucous coat to glide freely on the muscular in the movements and different degrees of contraction of the latter. In the submucosa the larger blood-vessels and some large nerves ramify.

Most internally is the **mucous coat**. This presents peculiarities in each part of the digestive tube, and consequently each part will require a separate description.

The mucosa constitutes nearly one-half the entire thickness of the wall of the tube, and in it are contained nearly all the glands of the stomach and intestine. The student must be careful not to confound the mucous membrane with the epithelium which covers it.

In the **œsophagus** the mucosa is formed of a rather dense fibrous tissue, similar to that which occurs in the mucous membrane of the mouth. It is covered by a compound scaly epithelium, into which some papillæ project. At its deeper part it contains the **muscularis mucosæ**. This is a layer of smooth muscular tissue, formed in the œsophagus of fibres arranged in bundles which run longitudinally. The muscularis mucosæ is an important structure, as it marks the deep limit of the mucous coat. **Mucous glands** occur in the œsophagus: scanty in man; very numerous in the dog; their acini lie mainly in the submucous coat. The ducts, which are lined by a low columnar, or, in the dog, almost flat epithelium, perforate the muscularis mucosæ and the mucous membrane, and open by narrow mouths on the surface.

The blood-vessels form a plexus around the glands, and another under the epithelium, and send loops into the papillæ.

At the cardiac orifice of the **stomach** the transition from the mucous membrane of the œsophagus to that of the stomach is abrupt, and marked by a line distinctly visible to the naked eye. The mucous membrane of the stomach can scarcely be said to have any general surface, for this is limited to ridges separating **pits, or depressions** (stomach tubes), which lie close together, and pass down vertically into the membrane for about one-fourth of its depth. These pits and the ridges between are lined by a single layer of high columnar epithelial cells, each containing near its attached end an oval nucleus. When the stomach is empty these cells are closed at their free ends; but during digestion they almost all undergo mucous transformation, or become goblet cells. Underneath this epithelium is a basement membrane, formed apparently of flat cells.

Into the bottom of each of the pits there open two, or three, or more **glands,** in the shape of tubes which pass downwards, and terminate by blind extremities just above the muscularis mucosæ. The pits may be looked on as the ducts of the glandular tubes which open into them. Each gland is narrowest for a short distance after it leaves the pit, and widens at its deeper part. The narrow part of the gland is called the neck; the deeper the fundus. The glands seldom branch, but are frequently bent near their closed ends. Each gland is formed of a continuation of the basement membrane of the pit, strengthened on its inner surface by flat stellate cells, similar to those described in the acini of the salivary glands. The **epithelium** consists of two kinds of cells—1. A continuous layer of polygonal, finely-granular cells, with round or oval nuclei. These cells are small in the neck, and become larger in the fundus. They are called **chief cells,** or central cells. 2. Cells oval in shape, coarsely granular, and not forming a continuous layer, but occurring at intervals, and always lying external to the chief cells,* often in depressions in the basement membrane. They are very numerous in the neck of the glands, but are more scattered in the fundus. They are called **parietal cells.** Their former name, 'peptic cells,' is now rightly abandoned. The central space, or lumen of the gland, is extremely narrow.

The glands which have been described are found in all parts of the mucous membrane of the stomach from the cardiac orifice to within a few inches of the pylorus. In this limited **pyloric region,** however, the structure of the mucous membrane undergoes a change. It is smoother, firmer, and more closely attached to the subjacent coats than is the case elsewhere. The pits, or **ducts,** into which the glands open are much longer than those at the cardiac end, and reach to more than one-half the depth of the mucous membrane. The **glands,** which open into these ducts in groups of three or four, are short, and rather flask-shaped than tubular, and frequently branched. The epithelium which lines the ducts does not differ from that which lines the ducts of the glands at the great end of the stomach ; but the pyloric glands are lined by a single layer of columnar cells, whose protoplasm is granular, and never undergoes mucous change, and whose nuclei are flattened, and lie near the outer attached ends of the cells. The nuclei, when seen in profile, appear crescentic, the convexity being turned towards the basement membrane.

These cells, although commonly stated to resemble the chief cells of the glands of the cardiac end of the stomach, differ from them both in their own shape and in that of their nuclei, and resemble much more closely the cells which line the tubes of Brunner's glands in the duodenum. As a rule there are no cells in the pyloric glands which correspond to the parietal cells of the glands of the cardia, but sometimes, particularly in preparations made with osmic acid, cells are found which have some resemblance to them. They are, however, most probably of a different nature, and merely a stage in development of the ordinary columnar cells of the part.

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* In the neck of the gland, where the tube is narrow, the parietal cells sometimes project through the chief cells, and help to bound the narrow lumen.

Between the cardiac and pyloric regions is a narrow zone, in which both kinds of glands occur together, and in which transitional forms are met with.

Corresponding to the differences in their structure, the secretion of the cardiac end of the stomach differs from that of the pylorus. Both contain pepsin; but the former is limpid and acid, the latter viscid and alkaline. From this it is assumed that the parietal cells of the cardiac glands are concerned in the secretion of acid. The mucus, which is abundantly formed in the stomach, is produced by the goblet cells, which cover the general surface, and line the pits or ducts.

The greater part of the mucous membrane is occupied by the glands. The intervals between them and the space below their closed ends is formed of a loose **connective tissue,** in which a variable number of **lymph corpuscles** is found. In some places in the deeper parts of the mucosa, the lymph corpuscles are accumulated, and the intervening tissue becomes a reticulum. These spots are called the **lymphatic follicles** of the stomach. They are inconstant objects.

The **muscularis mucosæ** consists of two or three layers of muscular fibres. It sends up vertical bundles between the glands. The **muscular coat** has, at the great end of the stomach, a layer of oblique fibres internal to the circular and longitudinal layers. The **pyloric valve** is formed by a great thickening of the circular layer of the muscular coat.

The **blood-vessels** form a plexus, with elongated meshes in the muscular coats. Large vessels ramify in the submucosa, from which branches pass into the mucous membrane. Here they pass upwards between the glands, forming a close plexus of capillaries, with meshes elongated in the vertical direction. This passes into a horizontally-disposed network of larger capillaries, which surround the openings of the ducts, just beneath the surface. From this the veins come off and pass downwards into the deeper parts.

The lymphatics form a plexus in the submucosa, and a finer plexus in the deep part of the mucosa between the glands. From this branches pass up between the glands, and end blind, or by anastomosing with one another just below the surface.

The **nerves** will be subsequently described.

The mucous membrane of the **small intestine** has, to the naked eye, a velvety appearance. This is due to a vast number of minute hair-like processes which project from the general surface of the membrane. These processes are called **villi.** They are small at the commencement of the duodenum, attain their greatest magnitude in the jejunum, and become small and few at the lower end of the ileum. Each villus is of a cylindrical or conical shape. It is covered by a simple columnar epithelium. The cells composing this epithelium have already been described. They have a striated border on their free end, a finely granular protoplasm, an oval nucleus, and are commonly attached to the basement membrane by a bent beak-like process. Goblet cells occur among them in variable numbers. In the axis of the villus runs a lymphatic vessel—the chyle vessel. External to this are muscular fibres derived from the muscularis mucosæ, and arranged for the most part parallel to the long axis of the villus. They are inserted into the basement membrane. Outside these, and lying just under the basement membrane, is a plexus of blood-vessels. The artery enters at one side, the vein leaves at the other, and between the two is a network of capillaries. All these structures are embedded in the tissue of the mucous membrane of which the villus consists, and of which it is merely a projection. This tissue is not fibrous, but retiform. In the reticulum flat endotheloid cells occur, and numerous lymph corpuscles. In fact, it is adenoid tissue, in which the reticulum is less fine and the corpuscles less numerous than in a lymphatic gland.

Between the bases of the villi there open the mouths of tubular glands, each of which runs an unbranched course through the thickness of the mucous membrane, and terminates by a closed extremity just above the muscularis mucosæ. These are **Lieberkühn's glands.** They are so numerous as to occupy almost the whole mucous membrane, as the glands of the stomach do. They consist of a basement membrane continuous with that of the surface and a single layer of epithelium, which, except for the less height of the cells, does not differ from that covering the villi and the general surface of the intestine.

The only other secreting glands to be found in the intestine occur in the upper part of the duodenum. These are **Brunner's glands,** and, unlike the other glands of the stomach and intestines, they are situated, not in the mucous, but in the submucous coat. The duct of each gland is lined by a simple columnar epithelium, and opens on the surface or into one of Lieberkühn's glands. Having perforated the muscularis mucosæ, it divides, in the submucosa, into a number of tubes, which branch and run a tortuous course, giving off a number of diverticula, which end by blind extremities. These tubes are lined by epithelium precisely the same as that which occurs in the pyloric glands of the stomach. Transitions between these latter and Brunner's glands exist; the pyloric glands becoming gradually longer and perforating the muscularis mucosæ. The ducts, however, differ in the two cases. In the pyloric glands they are wide pits, lined by goblet cells. In Brunner's glands they are narrow tubes, of less diameter than the proper gland-tubes, and the epithelium is not composed of goblets.

Throughout the small intestine there are to be found rounded or flask-shaped **masses of adenoid tissue,** in which the lymph corpuscles are far more numerous than in the general mucosa, and whose limits are pretty sharply defined. These resemble precisely the follicles of the tonsil, or those at the root of the tongue. They occur either singly or in groups. The former are the **solitary glands**, the latter the **agminated glands, or Peyer's patches.** Each follicle occupies both the mucous and submucous coats, and consequently perforates the muscularis mucosæ. It terminates below by a rounded base, and usually projects by a conical summit slightly above the general level of the mucosa. This summit is covered by epithelium. Where the follicle occurs there are no villi, and Lieberkühn's glands are pushed aside, and assume an oblique direction. In structure the follicle is identical with those which have been described in the mouth and tonsil. It is abundantly supplied with blood-vessels, and surrounded by a lymphatic plexus or lymph-sinus.

The solitary glands occur scattered irregularly. The agminated glands occur, as a rule, only in the ileum, where they form oval patches, situated always on the side of the intestine opposite to the attachment of the mesentery, and having their long axis parallel to that of the intestine. Each patch is formed of an immense number of follicles, lying closely side by side, and frequently becoming confluent where they touch one another.

There are several follicles at the commencement of the duodenum. In some animals the intestine is here surrounded by a ring of adenoid tissue.

The **muscularis mucosæ** of the small intestine consists, in most places, of two layers—an inner, circular; an outer, longitudinal. It sends bundles into the mucosa between Lieberkühn's glands, and into the villi.

The other coats call for no special notice.

The larger **blood-vessels** ramify in the submucosa. A close plexus surrounds Lieberkühn's glands, and communicates with the vessels of the villi. Brunner's glands have a special capillary plexus.

The central **chyle-vessels** of the villi are lymph capillaries, such as have been already described. Their wall is formed of a layer of endothelial cells, with very sinuous outlines, and they have no valves. They communicate with spaces in the tissue of the villi, which are the juice-canals, and it is through these that matters reach the central chyle-vessels, but it is not certainly known how the juice-canals communicate with the cavity of the intestine.

The **lymphatics** from the villi communicate with a plexus which surrounds the openings of Lieberkühn's glands. From this, branches pass to the deeper parts of the mucosa, where a second plexus exists. This communicates with a coarse plexus of large lymphatics in the submucosa, which have proper coats and possess valves. The lymphatics surrounding the follicles communicate with the others, which, together with those of the muscular coats, all pass to the edge of the intestine, where they enter the mesentery.

In the **large intestine** we find no new structures. There are no villi. **Lieberkühn's glands** are larger than in the small intestine, and many of the epithelial cells, both of the glands and of the general surface, are goblets. **Solitary follicles** are numerous and large. Corresponding to each of them there is a depression in the mucous membrane, into the bottom of which the follicle projects.

In the human colon the longitudinal layer of the **muscular coat** forms three bands, between which it is very thin or deficient.

In the rectum the muscular coat is very thick, and at the lower part contains some striated fibres.

At the anus the columnar epithelium passes into the compound scaly epidermis. There is a narrow zone of transitional epithelium between the two.

Around the anus is a ring of very large sweat glands, called the **circumanal glands.**

The **blood-vessels** of the large intestine resemble those of the stomach.

The **lymphatics** form a plexus just under the surface of the mucous membrane, and another in the submucous tissue.

In the walls of the stomach and intestines there exist two remarkable nerve plexuses, one lying between the longitudinal and circular layers of the muscular coat, and called the **myenteric plexus,** or **plexus of Auerbach;** the other situated in the submucous coat, and called the **plexus of Meissner.**

Each of these plexuses consists of nerve fibres, which are exclusively non-medullated. In Auerbach's plexus the fibres run in flattened bundles, surrounded by a distinct sheath, or perineurium, lined with endotheloid cells. The bundles form a very beautiful and regular fundamental network, with polygonal meshes. At the nodal points of this network are groups of ganglionic cells. Each cell is flattened, branched, and contains a single nucleus. From the fundamental network finer bundles come off, and form in its meshes a still closer secondary network, connected with which there are (at least in mammals) no ganglionic cells.

Auerbach's plexus communicates through the internal muscular coat with Meissner's plexus. This, situated in the submucous coat, is less regularly formed than is the myenteric plexus. Its bundles of fibres are thinner, and their meshes are less regular in form and size, and do not all lie in a single plane. The ganglia are not so flattened, and the individual cells are rounder.

The ganglionic nerve-cells belonging to these two plexuses are very numerous—probably not less in number than those contained in the spinal cord.

The **myenteric plexus of the œsophagus,** in that part where the muscular coat consists of striated tissue, differs from the corresponding plexus in the intestine. It has been well studied by Ranvier. There is a network of non-medullated nerve fibres, with ganglia. The meshes of the network are larger and more irregular, and the ganglia are larger and contain larger nerve-cells than are to be found in Auerbach's plexus in the intestine. The chief difference, however, consists in the presence of medullated nerve-fibres, which are derived from the pneumogastric, and which, after dividing and subdividing, and entering into connexion with the non-medullated fibres, terminate in the striated muscle by numerous end-plates, such as have been described as the terminal organs of motor nerves in the higher animals. According to Ranvier, the connexion of the medullated fibres with the ganglia is similar to that between the fibres of a spinal nerve-root and the cells of the spinal ganglia. A nerve cell sends a fibre (here non-medullated), which joins in a T-shaped figure, with a medullated fibre, at a Ranvier's constriction.

A plexus, corresponding to Meissner's, exists in the submucosa of the œsophagus. The details of its arrangement have not been particularly studied.

There can be no doubt that the myenteric plexus is mainly concerned in regulating the movements of the digestive canal. The function of Meissner's plexus is uncertain. It may be to regulate the secretion of the glands, or to supply the muscularis mucosæ and the blood-vessels of the mucous membrane.

The digestive tube may be hardened in chromic acid, or the chromic salts, but strong alcohol alone gives good results, and has the advantage that the sections stain quickly and well, which is not the case when the parts have been acted on by chromium compounds.

1. **Œsophagus** of a dog cut into short pieces, and hardened in alcohol. Vertical sections through the coats, cut accurately either transversely or longitudinally; stained in logwood; mounted in Canada balsam or dammar.

The different layers of which the tube consists may be seen with a lens, or very low power of the microscope. The details of each must be studied with the high power. Observe the compound scaly epithelium; the firm fibrous structure of the mucosa; the muscularis mucosæ, composed of smooth muscular tissue, and not forming a compact layer, but consisting of bundles cut across if the section be transverse; in length if longitudinal: below this the acini of the mucous glands, their ducts passing to the surface, where they open each by a narrow mouth; the loose connective tissue of the submucosa, containing blood-vessels; the two muscular layers, consisting, in the upper part of the œsophagus, of transversely-striated fibres, with which in the lower part smooth fibres are mixed. If the section be transverse, the fibres of the internal layer are cut in length; those of the external are divided across; if longitudinal, the opposite is the case. Hence the direction of the section may be known by the appearance of the muscular coat. This is true for the whole intestine.

2. Vertical sections through the coats of the cardiac end of a dog's **stomach,** hardened in alcohol, stained in logwood or cochineal; best of all, in logwood and eosine, which stain the chief cells blue, and the parietal cells bright red. The section should be very thin, and mounted in glycerine or balsam.

The muscular coat has an irregular appearance, varying according to the direction of the section. The submucosa, composed of loose areolar tissue, contains no glands. In the mucous coat notice the short depressions passing down from the free surface and the ridges between them; the high columnar, often goblet cells which cover these parts. From the bottoms of these depressions the long tubular glands passing down to just above the muscularis mucosæ. These glands run a somewhat sinuous course, and are consequently often not seen in their entire length. Their lumen is very narrow, and will not be visible if the section passes at one side or the other of the axis of the tube. Observe the continuous lining of the gland by—(1) polygonal cells, with round nuclei (chief or principal cells). These cells stain in logwood and cochineal better than the—(2) large oval granular cells (parietal cells), which lie outside them. These, on the other hand, stain intensely red in eosine. They are numerous at the upper part of the gland, more scattered below, and are often seated in depressions of the basement membrane.

Observe the tissue of the mucous membrane between and below the glands. It contains numerous lymph corpuscles, which are sometimes crowded together to form a follicle. The muscularis mucosæ consists of two or three layers, and sends bundles of fibres between the glands.

In the submucous and deeper parts of the mucous coat numerous blood-vessels will be seen.

3. Sections through the mucous membrane of the cardiac end

of the stomach, parallel to the surface, and made at different depths, stained as above, and mounted in glycerine or balsam. (*a*). Near surface. Cross section of pits or ducts, which appear as round spaces, lined by columnar (goblet) cells. (*b*). A little deeper, through necks of glands. These are seen as groups of small round figures, with a very narrow lumen, and lined by small pyramidal cells (chief cells), with several large oval cells (parietal cells) lying external to these, and sometimes between them. (*c*). Deeper, through the fundus of the glands. The glands are larger; the lumen, although small, is distinct; the chief cells are pyramidal, the bases external; most glands present one or two parietal cells, but some may want these entirely, owing to the more scattered disposition of the parietal cells in the deeper parts of the glands.

4. Vertical section through the coats of the **pyloric portion** of the stomach. The pits, much longer than at the cardiac end, are lined by columnar goblet cells. From the bottom of these come off glands, narrow at their origin, subsequently becoming wider, and which branch and spread out horizontally. Consequently, many are separated from the pits to which they belong, and are seen in cross section. Their epithelium consists of long columnar cells, with crescentic nuclei near the attached end. The interglandular tissue is here more abundant than at the cardiac end. It is not necessary to make horizontal sections, as the pits do not differ from those of the fundus, except in their length, and many of the glands are seen cut across in vertical sections.

5. Longitudinal section through the **junction of œsophagus and stomach.** The vessels injected with Prussian blue. Stained in cochineal; mounted in Canada balsam. The muscular and submucous coats and the muscularis mucosæ pass continuously from the œsophagus into the stomach. There is no considerable thickening of the circular muscular coat, no true cardiac sphincter. The transition from the glandless mucous membrane of the œsophagus, with its papillæ and compound scaly epithelium, to that of the stomach, with its glands and columnar epithelium, is abrupt. The loops of capillary vessels in the papillæ of the œsophagus and the plexus surrounding the gastric glands will be seen.

6. Longitudinal section through the coats of upper part of **duodenum** of dog or cat. Stained in logwood, or logwood and eosine; mounted in balsam. External muscular coat cut in length. Internal muscular coat much thicker, and cut across, fibres appearing as small round or polygonal figures, with or without nuclei; muscular tissue traversed by connective tissue bands, with blood-vessels. Submucosa contains Brunner's glands. In carnivora these are limited to a narrow region just below the pyloric valve. Observe that they lie below the muscularis mucosæ, which is perforated here and there by a duct. Notice the groups of rounded figures, the cross sections of the

tubes, lined by epithelium identical with that occurring in the glands of the pylorus. Observe the villi projecting from the surface of the mucosa. Lieberkühn's glands extend from the surface between the villi to the muscularis mucosæ, which consists of two layers. A lymph follicle will probably be seen in the mucosa. Observe its similarity to those of the tonsil.

7. Section longitudinally through **junction of stomach and duodenum.** Logwood and eosine; balsam. Observe the great thickening of the circular muscular coat (pyloric valve), appearing in cross section as a triangular figure, the apex internally. Over this pass the mucous and submucous coats. On the duodenal side observe the appearance of villi, the lymph follicle, the gradual extension of the pyloric glands through the muscularis mucosæ to form the glands of Brunner. The muscular tissue is red, the nuclei blue.

8. Longitudinal or transverse section of **jejunum.** Logwood; balsam. Observe the great length of the villi. Some of the villi may not have smooth outlines, but present transverse constrictions and bulgings. This is due to the contraction of their muscular fibres which occurs when the living tissue is placed in the hardening fluid. Observe in the villi the epithelium, with scattered goblet cells; the lymphoid retiform tissue of which the villus is composed, and the elongated nuclei of the muscular tissue and of the blood-vessels. Lieberkühn's glands as in duodenum. Other coats as before.

9. Horizontal section of mucous membrane of jejunum. Logwood; balsam. Lieberkühn's glands cut across, appearing as round figures, with distinct lumen, surrounded by columnar epithelium, mixed with goblet cells. Interglandular tissue a reticulum, with numerous lymph corpuscles.

10. Section through **Peyer's patch** of dog. Logwood; balsam. Muscular coats as before. Numerous lymph follicles, some appearing to lie altogether in the submucous coat, others perforating the muscularis mucosæ and extending to a variable distance into the mucosa. This difference depends on the part of the follicle which is cut. Each follicle is flask-shaped, its broadest part lying in the submucosa. If the section be made through the periphery of the follicle, this will appear to lie altogether below the muscularis mucosæ; but if nearer to the axis, it will extend into the mucosa also. Where a follicle reaches the surface, it projects as a high conical elevation, covered by columnar epithelium. Observe how Lieberkühn's glands are pushed aside by the follicles. The villi are small, and are absent over the follicles.

In the cat and dog the lower part of the ileum is occupied by a continuous layer of lymph follicles.

11. Vertical section through the wall of the **large intestine** of a dog. Logwood; balsam. Villi absent. Lieberkühn's glands large. Epithelium in dogs contains numerous large goblet

cells. Solitary lymph follicles of large size, readily visible to the naked eye; flask-shaped, and lying in mucous and submucous coats; over each a depression in the mucous membrane where Lieberkühn's glands are absent. Other coats as before.

12. Section through coats of small intestine, whose blood-vessels have been injected. In the muscularis the vessels form a network, with meshes parallel to the direction of the fibres; consequently, if these are cut in length, so will most of the blood-vessels, and, on cross-section of the muscular fibres, the vessels will be mostly cut across.

From the large vessels in the submucosa branches pass into the mucosa, and form a plexus about Lieberkühn's tubes and in the villi. Observe that in the villi the vessels lie near the surface, leaving the axial part free.

Injected duodenum will show a plexus about Brunner's glands, and in the ileum the plexus belonging to the lymph follicles will be seen.

13. Section of small intestine whose lymphatics (lacteals) have been injected by puncture. A lymphatic vessel occupies the axis of each villus, and opens into a plexus situated under the surface of the mucosa, and surrounding the openings of Lieberkühn's glands. Between the latter, vessels descend and go to a coarser plexus in the submucosa. Lymphatic vessels are seen also between the layers of the muscular coat.

14. **Meissner's** and **Auerbach's plexus** are best prepared by staining with chloride of gold, which is not suitable for class work. Preparations should be examined. Good views of Auerbach's plexus may be got by the method recommended by Orth. A piece of the intestine of a rabbit or guinea-pig is distended with dilute acetic acid (5 drops to 100 cc. water), the ends tied, and the distended intestine placed in the dilute acid for 24–48 hours. A shallow incision with a sharp scalpel is made through the external muscular coat, and with a fine-pointed pair of forceps large pieces of the longitudinal muscular coat may be detached, with Auerbach's plexus adherent. The pieces are washed in water; stained in logwood; carefully spread on the slide, and mounted in glycerine. The network of nerve fibres and the ganglia are stained blue, and can be well seen with a low power.

III.—*Liver.*

The bulk of the liver is formed of the **hepatic cells,** which are polygonal epithelial structures. They have no cell-wall; they possess each one, or sometimes two, round or oval nuclei. The protoplasm frequently contains fat, or glycogene, or pigment granules, besides other granules of an albuminoid character, and which become pale with acetic acid. In the protoplasm the appearance of a network is sometimes very distinct. These cells do not form a diffuse, uniform tissue, but are grouped into little masses, the hepatic lobules, whose outlines, as well as general arrangement, are determined by the distribution of the blood-vessels.

The liver receives blood from two sources—namely, the hepatic artery and the portal vein. The blood of both these vessels is conveyed away by the hepatic veins. The hepatic veins, in their course through the liver, run alone, and are surrounded by very little connective tissue, but are closely attached to the hepatic tissue, and consequently remain open, and do not collapse when cut across.

When the **hepatic veins** are followed to their smaller divisions, there can be seen through their thin walls a polygonal network, formed by the outlines of the bases of the hepatic lobules, which are closely attached to the exterior of the vessels. These small hepatic veins, as they run in immediate apposition with the bases of the lobules, are called **sublobular veins**. From them there come off, nearly at right angles, minute branches, each of which enters into the interior of a lobule, and is consequently called an **intralobular vein.**

A **hepatic lobule** has, roughly stated, somewhat the shape of a conical bullet; its base rests on the sublobular vein, and it is through the base that the intralobular vein penetrates the lobule, and in its axis that it runs. It does not, however, run the entire length of the lobule, but at a certain point it breaks up into a system of capillaries, which have a general radiating course towards the periphery of the upper or conical portion of the lobule. Before breaking up finally into these capillary vessels, the hepatic vein, from the moment of its entrance into the lobule, gives off from its sides a number of capillaries, which all diverge in a radial direction towards the sides of the lobule. All the capillaries of the lobule, although they run on the whole a radiating course, branch and anastomose freely, so that the lobule is occupied by a close capillary plexus, with radially-disposed meshes, and the centre of which is the intralobular vein.

The **portal vein** enters the liver at the portal fissure, in company with the hepatic artery and duct. These three vessels branch together, and run in company down to their very finest ramifications, and constitute what are called the **portal systems.** The spaces in which they lie are called portal canals. They are always surrounded by a considerable quantity of loose connective tissue, which allows the large thin-walled portal vein to collapse when it is cut across. The connective tissue which ensheaths the structures of a portal system is called the **capsule of Glisson.** The terminal branches of the portal veins still enclosed in this capsule reach the outer side of the lobules. They must, therefore, be placed between neighbouring lobules, and are consequently called **interlobular veins.**

In most animals the connective tissue is limited to the immediate neighbourhood of the interlobular veins, and appears in sections only as little triangular-shaped patches, lying at intervals about the circumference of the lobules. In some few animals, however, of which the pig is an example, each lobule is completely enclosed by a fibrous capsule, which is continuous with the connective tissue surrounding the portal vessels, or the capsule of Glisson.

The interlobular veins having reached the periphery of the lobules, break up into **capillaries,** which, entering the lobules on all sides, run in each lobule centripetally towards the intralobular vein. These are the capillaries which have been already described as forming the radially-disposed network in the lobule. They, therefore, communicate between the interlobular portal vein and the intralobular hepatic vein. The intervals between these capillaries are filled by hepatic cells, which lie closely together; and as the material which fills up the interstices of a network which is extended in all directions must itself form a network, the masses of hepatic cells in each lobule form a network, with elongated, radially-disposed meshes. The network of vessels is so close, that each hepatic cell is in contact with at least one, and generally with several capillaries.

The **hepatic artery** breaks up into capillaries in the portal canals, and supplies the connective tissue, the walls of the portal veins and of the bile ducts. From these capillaries are formed veins (internal roots of the portal vein), which again break up into capillaries, and join the capillary system of the lobules. A direct anastomosis of primary or arterial capillaries of the hepatic artery with the lobular capillaries takes place to a very limited extent only. Hence it will be seen that, although the blood entering the lobule comes from two sources, yet it is all venous—the portal blood having become venous in the stomach and intestines, the blood of the hepatic artery in the portal canals.

From the account which has been given of the shape of the hepatic lobule, and of the arrangement of parts in it, it is clear that the appearances must differ greatly according as a section goes through the axis of the lobule or at right angles to this. In the first case the lobule appears as as an elongated figure, the intralobular vein is cut in length, and its capillary branching is evident. In the second, the lobule has a roundish shape, with the intralobular vein cut across in its centre, from which the blood-vessels and hepatic cells radiate towards the periphery.

The limits of each lobule can admit of no doubt in those livers in which the lobules are enclosed in a fibrous sheath, but in the human liver and that of most mammals the outlines of the lobules are very badly defined. Taking the lobules as rounded bodies lying in contact with one another, it is clear that there must be triangular-shaped intervals between them. In these intervals there appear the terminal divisions of the portal vein, hepatic artery, and hepatic duct; but in the interspaces between these terminal portal systems there is nothing to separate one lobule from another, except that at the boundary line, the centre from which the cells and capillaries appear to radiate alters. The radiation is more distinct near the centre than at the periphery of the lobule; and if the section fall obliquely, or so as not to include the intralobular vein at all, the boundary of the lobule can only be drawn by an imaginary line connecting together the different portal systems which lie at its periphery.

The intralobular vein is the most important landmark, since it fixes the centre of the lobule. It may always be readily distinguished by its lying alone, and having only a very minute quantity of connective tissue about it, while the portal veins are always accompanied by the hepatic arteries and bile ducts, and surrounded by the capsule of Glisson.

The walls of the larger **bile ducts** are composed of connective tissue, with a considerable number of smooth muscular fibres arranged transversely and obliquely.

The epithelium consists of high columnar cells, with a striated border, like that in the intestine. There are numerous mucous glands opening into the larger ducts, while the smaller ducts have branching diverticula, which probably serve as glands. The finer interlobular ducts have a thin connective tissue wall, with scattered muscle cells and a cubical epithelium. They branch freely, and form a network which surrounds the lobules.

The intralobular ducts, or **bile capillaries,** form a network surrounding the hepatic cells. Whether they are merely fissures between the cells, and bounded only by them, or whether they have a proper wall, is still a doubtful point, as is the exact mode in which they communicate at the periphery of the lobules with the interlobular ducts. In the interior of the lobules the bile capillaries and the blood capillaries are always separated by at least half the diameter of a hepatic cell. The blood capillaries are generally situated where the angles of several hepatic cells meet, while the bile capillaries run between the apposed surface of the cells. This arrangement, however, is by no means constant.

The **gall-bladder** is lined by columnar epithelium, with a striated border. The mucous membrane has numerous folds on its inner surface, which gives it a honeycomb appearance. There is a muscular coat mixed with much connective tissue, the whole being covered by peritonæum. The blood-vessels of the gall-bladder and of the larger bile ducts are numerous, and derived from the hepatic artery. There are some acinose mucous glands in the wall of the gall-bladder.

The liver is enclosed in a dense **fibrous capsule,** from whose deep surface processes pass into the organ between the superficial lobules, and which is continuous with the connective tissue of the portal canals at the transverse fissure. Outside the proper capsule is the peritonæum.

The **nerves** of the liver are derived from the cæliac plexus, and in part directly from the right pneumogastric. The fibres are mostly of the non-medullated variety, and are probably for the supply of the blood-vessels and ducts. Whether any direct connexion exists between the secreting cells and the nerves is unknown. Ganglia are said to exist in the walls of gall-bladder and larger ducts.

The lymphatics of the liver are superficial and deep. The former run in the capsule, where they form a network; the latter in the portal canals. In the interior of the lobules an interval can frequently be seen between the capillaries and the hepatic cells, which is considered to be a lymphatic space; so that the capillaries are supposed to lie in a perivascular lymphatic. Traversing these lymphatic spaces are a few branched connective tissue cells.

The lymphatics of the gall-bladder join, in the portal fissure, with the deep lymphatics from the liver.

1. A section is made into the liver of a recently-killed animal, dog, rabbit, &c. The surface of the section is gently scraped with a scalpel, and the scraping diffused in a drop of salt solution on a slide. On examination with a high power there are seen, besides numerous blood corpuscles, the liver cells, large, polygonal, granular bodies, often containing fat and pigment granules.

Irrigate with acetic acid, when the cells will become less granular, and the nuclei will become more distinct.

2. Pieces not larger than a cubic inch of the liver of a pig are suspended in a large quantity of Müller's fluid, which is changed every day for the first three or four days. After about four weeks the pieces are removed, washed in water, and placed in alcohol, which should be changed after twenty-four hours.

Sections, which should be of a good size, and which are consequently best made with some form of microtome, are stained in logwood, and mounted in balsam.

With a very low power the section is seen to be divided into spaces by clear lines, which form a network. The spaces are the lobules; the clear lines are the connective tissue capsules, which in the pig surround and separate the lobules. The spaces have not an uniform size and shape—some are elongated; others more roundish, or evenly polygonal. This is due to the fact that the lobules do not lie all in one direction, so that a section made in any plane cuts some in length, others across, others obliquely. In the centres of many of the spaces the intralobular veins are seen. When the spaces have an elongated shape, and are consequently cut in length, the vein appears as a long figure. If the lobule is cut across, the vein appears round. In either case it is seen that the vein is unaccompanied by any other vessel, and that it is surrounded by little or no connective tissue. In some lobules two intralobular veins may be found. Much more commonly lobules will be seen without any vein in their interior. This is due to the section having passed either transversely through the lobule above the point, where the intralobular vein resolves itself into capillaries, or longitudinally or obliquely between the vein and the periphery of the lobule, so that the vein is not included in the section.

In those lobules in which the vein is visible it is seen that the hepatic cells lie in rows, which radiate from the vein, and have clear lines between them. These lines are the empty capillary vessels. When the section has gone at right angles to this radiation, or, in other words, is parallel to the long axis of the lobule, the capillaries will be seen with a high power as minute round openings between the cells. Numerous blue dots cover the section of the lobule. These are of two kinds. The most of them are round : these are the nuclei of the hepatic cells. Others are spindle-shaped, or irregular, and more intensely stained than the round nuclei : these are the nuclei of the endothelium of the capillary vessels.

In the nodal points of the network of clear connective tissue lines which separate the lobules, that is, where several lobules meet by their angles, sections of the interlobular veins, arteries, and ducts are seen. The vein appears as a space in the tissue, without proper walls, beyond a delicate layer of spindle-shaped endothelium bordering its lumen. The artery may be recognized by the spindle-shaped nuclei of its muscular coat. When a small artery is cut transversely there appear in the centre a certain number of small, round, deeply-stained points. These are the nuclei of the endothelium, really spindle-shaped, but here cut across, and appearing round. Outside these, and, as it were, embracing them, are the curved, spindle-shaped nuclei of the circular muscular coat. If the artery be very small there may be only one muscle-nucleus, appearing as a crescentic figure, holding in its concavity two or three endothelial nuclei. The small arteries are generally so contracted that their lumen is not visible. If the artery be cut longitudinally or obliquely, it presents two sets of spindle-shaped nuclei, crossing each other at right angles. The endothelial nuclei are elongated in the direction of the tube ; the muscular nuclei lie across it, so that, however an artery is cut, it always has spindle-shaped nuclei in one or both directions.

The small ducts, on the other hand, are lined by a cubical epithelium, with round nuclei, and since the cells are of equal size, these nuclei lie at close and perfectly regular intervals. If the duct be cut across, they form a ring around the lumen, which, although small, is usually distinct. If the duct be cut obliquely or longitudinally, it presents a series of round nuclei perfectly evenly spaced, larger and less deeply stained than the cross section of the nuclei of the arterial endothelium, and practically unmixed with spindle-shaped nuclei, for the quantity of muscular tissue in the small ducts is so minute as not seriously to interfere with the diagnosis.

By attention to these points the distinction between the vein, artery, and duct may be readily made ; and it must be borne in mind, that in a single portal canal several examples of each kind of vessel may be met with, since they all branch freely.

If any of the larger portal canals occur in the section, the vessels in them will be readily recognized by their ordinary characters. Sections of lymphatic vessels are frequently to be seen in the portal canals.

If the section has gone through the fibrous capsule of the liver, it will be seen that this is continuous with the septa between the superficial lobules.

3. Section of a rabbit's or dog's liver hardened in Müller's fluid and alcohol; logwood: balsam. The outlines of the lobules are more distinct in the liver of the rabbit than in that of the dog or the human liver. In these the appearance are as if the fibrous tissue which surrounds the lobules in the pig's liver were all removed, except the little patches which ensheath the interlobular vessels. The intralobular vein and the parts in the interior of the lobule are as in the pig's liver; but at the periphery the lobules are confluent, and are separated by imaginary lines drawn from one interlobular system to another. Still by determining which vessels belong to the interlobular and which to the intralobular systems the outlines of the lobules can generally be easily defined, even when the vessels are not injected.

4. Section of the liver of a cat, rabbit, dog, &c., of which the vessels have been injected from the portal vein. If the injection be red (carmine), stain with logwood; if Prussian blue, stain in picrocarmine or cochineal; mount in balsam. In the lobules the capillaries are seen to form a dense plexus. They form elongated meshes, and converge towards the central vein. It may be seen how the interlobular vessels spread out their branches on the surface of the lobules, and how each vein supplies several lobules, while each lobule gets its blood on one side from one interlobular vein, and on the other side from another. With a high power it may be seen how the hepatic cells lie in the meshes of the capillary plexus, and the relation of the vessels to the cells.

5. In very thin sections of a well-hardened liver minute round openings may be seen with a high power in the lines separating neighbouring hepatic cells. Each opening is bounded by two semicircular depressions, one in each of the apposed cells. The openings lie not at the angles of the cells, but about the middle of the line joining two angles. These openings are cross sections of the intralobular bile capillaries.

Injection of the bile capillaries from the hepatic duct is difficult, and can never be more than partially successful, as the injection drives before it the contents of the ducts; but by introducing into the blood of an animal some coloured substance, which is eliminated by the liver, and killing the animal while this elimination is in progress, the bile capillaries may be filled by a natural injection. About as much indigo-carmine in powder as will fit on the point of a penknife is introduced under the skin of the thigh of a frog, and the wound closed. Twenty-four hours afterwards the animal is killed, and the liver placed in strong alcohol, which precipitates and fixes the indigo in the bile ducts. Fine sections, mounted in balsam, show the very minute canals filled with blue matter, and forming a networ around the cells. The intralobular ducts are very much fine than the capillary blood-vessels, and form a closer network, th meshes of which are never larger than the diameter of a singl hepatic cell. They are always separated from a blood-vessel b half the diameter of a cell.

6. Sections of a liver which has been hardened in Müller fluid only, without subsequent treatment with alcohol. Th sections are placed for some hours in osmic acid one pe cent., washed, stained in picrocarmine, and mounted in glycerine.

The osmic acid stains the fat globules in the cells black. the liver had been in alcohol the fat would have been dissolve out of the cells.

In moderately fatty human livers, such as are very commo it will be seen that the fat occurs only in the cells at the periphery of the lobules. In these cases the outlines of the lobule are beautifully defined by the fatty cells.

IV.—*Pancreas.*

The pancreas is an acinose gland, built on th same general plan as the salivary glands, fro which, however, it differs in many important particulars.

A connective-tissue capsule surrounds the glan and gives off septa, which, passing in, divide it int larger and smaller lobes and lobules, and sends small quantity of tissue into the spaces between th acini. The primary duct divides and subdivide until the divisions become extremely small. Thes terminal ducts open into long, tortuous, tubular dilatations, the acini, which are lined by the secretin epithelium.

The primary **duct** has a wall, consisting of connective tissue, lined by columnar epithelium, an has small mucous glands opening into it. In th smaller ducts the epithelium becomes lower, and a last is almost flattened. There is no part of th pancreatic duct with high striated epithelium lik the intralobular salivary tubes, but the passage fro the larger to the smaller ducts is gradual.

The walls of the **acini** are formed of a basemen nembrane, strengthened internally by flattened stelate cells. The **epithelial cells** are pyramidal, heir base being at the periphery, and their smaller nd turned towards the lumen of the acinus. Each ell consists of an outer part, which is homogeneous, r finely striated, and stains with carmine and logvood; and of an inner zone, which does not stain, nd which has a coarsely granular structure. The ound nucleus is situated at the junction of these wo parts. The appearance which the cells present aries a good deal, according to the stage of digestion n which the animal was at the time of the removal f the gland. This is due to the fact that the ranules which occur in the inner portion of the ells are formed of a substance which is used up in he formation of the secretion.

The flat epithelial cells of the finer ducts are ontinued for a certain distance into the acini, overing the secreting cells, and separating them rom the lumen. They are called **centroacinar ells**.

The **blood-vessels** form a plexus, which sur-ounds the acini with a close capillary network.

The **lymphatics** commence as sinuses about ach acinus. These open into lymphatic capillaries, nd these into larger vessels with valves.

The **nerves** run with the vessels. They consist mostly of non-medullated fibres, and are in laces connected with groups of ganglionic cells. Pacinian corpuscles occur in the pancreas of the cat.

1. Pancreas of dog or cat hardened in alcohol. Sections tained in logwood or carmine, and mounted in glycerine.

The general appearance of the section resembles at first sight hat of a salivary gland; but the absence of the salivary tubes, vhich are such prominent objects in the salivary glands, serves t once to make the distinction. Besides, in the pancreas the cini are more manifessly tubular, and their cells, consisting f two zones, differ from the secreting cells of any variety of alivary gland.

Observe the acini, with the cells, whose outer part, with the nucleus, is stained, but whose inner part is granular and without colour. In places centro-acinar cells may be seen in the interior of the acinus, whose lumen is always small. The small intralobular ducts, with flat epithelium, may sometimes be traced into an acinus.

In the connective tissue between the lobes some of the larger ducts, with high columnar epithelium, may be seen.

2. Injected pancreas; carmine or cochineal; balsam. A plexus of vessels surrounds the acini.

CHAPTER XII.

ORGANS OF RESPIRATION.

I.—*Larynx.*

The thyroid, cricoid, and the greater part of the arytenoid **cartilages** are formed of hyaline cartilage. The cells are flattened near the surface; deeper they are arranged in groups, which are commonly elongated and placed perpendicularly to the surface. The epiglottis, the apex and the vocal process of the arytenoid, and the smaller cartilages, are formed of elastic or reticular cartilage.

The inferior thyro-arytenoid and the middle crico-thyroid **ligaments** consist mainly of elastic tissue. In the other ligaments white fibrous tissue preponderates. The cartilaginous nodule in the posterior thyro-hyoid ligament is hyaline cartilage.

The **epithelium** of the epiglottis is compound scaly, thicker on the anterior than on the posterior surface. Deeper in the larynx this gradually changes to columnar ciliated, but on the edges of the true vocal cords and for some distance below these, as well as on the inner surfaces of the arytenoid cartilages, compound scaly epithelium is again found. Where this latter epithelium occurs, the mucous membrane projects into it in the shape of papillæ. The columnar ciliated epithelium has numerous goblet cells, and a narrow zone of transitional epithelium occurs where the columnar and scaly epithelia meet. The **mucous membrane** of the larynx is dense, and contains a considerable quantity of elastic tissue. Under the epithelium is a basement membrane, which becomes thicker in the deeper parts of the larynx. The mucous membrane contains, in places, considerable quantities of adenoid tissue. The **submucous tissue** is abundant on the anterior surface of the epiglottis and the false vocal cords; scanty in other places, as on the posterior surface of the epiglottis, the true vocal cords, and over the cricoid and thyroid cartilages. **Mucous glands** are numerous in most places, but are absent over the true vocal cords. Bodies similar to the taste-buds which occur on the fungiform and circumvallate papillæ of the tongue, exist in large numbers in the epithelium on both surfaces of the epiglottis and on the ary-epiglottidean folds; also in the deeper parts of the larynx, even on the edges of the vocal cords (Simanowsky).

The **blood-vessels** are numerous, and form a plexus in the superficial parts of the mucous membrane, sending loops into the papillæ where these are present.

The **lymphatics** form plexuses in the mucous membrane and submucous tissue.

The **nerves** are numerous. Ganglia are said to occur on the posterior surface of the epiglottis. Some of the nerves terminate in end-bulbs, others in the taste-buds, and others end in the epithelium, either in brush-like groups of fine axis cylinders, or in a network (Simanowsky).

II.—*Trachea and Bronchi.*

The rings of the **trachea** and **primary bronchi** are formed of hyaline **cartilage** of precisely the same character as that found in the larynx. They are covered by a dense **perichondrium**, which is continued from one ring to the next, and in the interspaces between the rings constitutes the outer or **fibrous layer** of the trachea. Posteriorly, extended across the space where the rings are deficient, is a membrane composed of smooth muscular fibres **(trachealis muscle)**, inserted at each end into the extremities and inner surface of the cartilaginous rings. Outside the transverse fibres there are some longitudinal bundles, and the whole is enclosed in an extension of the fibrous tissue which encloses the cartilaginous rings. The **epithelium** is columnar ciliated, with numerous replacement cells and goblets. In the dog the epithelium of the posterior wall of the trachea, or that part where cartilage is wanting, is in many places non-ciliated and of a transitional character. The epithelium rests on a homogeneous basement membrane, which is peculiarly thick in the human trachea. The **mucous membrane** contains a very large quantity of longitudinally-disposed elastic tissue and numerous lymph corpuscles. It is not sharply separated from the submucosa, as is the case in the intestine, but passes gradually into a loose tissue, in which lies an almost continuous layer of acinose **mucous glands**, whose ducts open by trumpet-shaped mouths on the surface. The glands are often deficient over the most prominent part of the cartilaginous rings. Posteriorly, where the submucous tissue is scanty, the glands occur not only internally, but externally to the muscular layer, and among the bundles of this. Between the trachea and œsophagus is loose connective tissue, in which, as in the posterior wall of the trachea, microscopic **ganglia** are often met.

The blood-vessels, lymphatics, and nerves call for no special notice.

The primary **bronchi** resemble the trachea, but as the air tubes become smaller by division, their structure changes. Their wall consists externally of a layer of **connective tissue,** in which are embedded irregular-shaped pieces of cartilage, which become smaller and less numerous as the size of the tube diminishes. Internal to this is a layer of **muscular tissue,** which completely encircles the bronchus, as the muscular coat does an artery. This explains the serious symptoms which may result from a spasm of the muscular coat, by which the bronchus can be narrowed to a very considerable extent. Inside the muscular layer is the **mucous membrane,** which contains a very large quantity of longitudinally-disposed elastic tissue, and is thrown into a number of longitudinal ridges, so that on cross section the lumen of the tube has a stellate shape. These ridges are persistent, but are made more prominent by a contraction of the muscular coat. The **epithelium** is columnar ciliated, with goblet cells, and rests on a hyaline basement membrane. There are numerous mucous glands whose acini lie in the outermost connective-tissue coat, and whose ducts pass through the other layer to open on the surface. The ducts frequently present ampullary dilatations, and are in part lined by ciliated epithelium.

When the bronchi become less than one millimetre in diameter, they are called **lobular bronchi,** since each pulmonary lobule receives one such bronchus. They come off from the larger bronchi nearly at right angles, and their origins follow one another in a spiral line. They have neither cartilage nor glands. Their wall is composed of **connective and elastic tissue,** with some **smooth muscular fibres.** The **epithelium** is ciliated and columnar, the cells progressively diminishing in height as the tube becomes smaller.

These bronchi divide dichotomously, the divisions standing to one another nearly at right angles. Each terminal bronchus, or bronchiole, which has a diameter of about one-third of a millimetre, ends in a pulmonary acinus or system of alveolar passages.

III.—*Air Spaces.*

A **pulmonary acinus** is a somewhat conical-shaped structure, which receives the bronchiole at its apex. It consists generally of three or more tubes **(alveolar passages),** which diverge from one another. They are not cylindrical, but their wall is thickly occupied by hemispherical or polygonal diverticula **(alveoli),** which open into the alveolar passage, but do not communicate directly with one another. Each alveolar passage terminates in a certain number of somewhat conical-shaped sacs **(infundibula)**, whose walls are covered by alveoli; and similar infundibula open off from the sides of the alveolar passages before the termination of the latter.

These infundibula and alveoli, which are the ultimate air spaces of the lung, are lined by a **flat epithelium,** consisting of a single layer of cells, which meet by their edges. Two kinds of cells occur—one small and granular, and with a distinct nucleus; the other large, very thin, with indistinct nuclei. The cells of this latter kind, which are the more numerous, are probably derived from the former by a process of mechanical extension in the movements of respiration. The epithelium rests on a homogeneous **connective tissue**, containing cells, with oval nuclei. This is mixed with a quantity of **elastic tissue**, and a few **muscular fibres,** which are most numerous where the bronchus passes into the acinus. They occur also at the openings of the infundibula and of the alveoli, but in the walls of the latter no muscular tissue exists.

Before the bronchi open into the acini, they present for some short distance a gradual change of their epithelium, from the columnar ciliated epithelium of the larger tubes to the mixed large and small flat cells of the alveoli. On the walls of these transitional tubes a few alveoli are met with (Kölliker).

The lung is traversed by tracts of connective tissue, which enter at the hilum with the bronchus and large blood-vessels, and which at the periphery become continuous with the subpleural tissue. This connective tissue accompanies the bronchi and larger vessels in all their ramifications, and circumscribes groups of from two to twenty acini, which groups are called **lobules.** In the human lung the outlines of the lobules at the surface are usually very distinct, being marked out by black lines, formed by the smoke and other carbonaceous matter which has been inhaled during life, and which has found its way through the walls of the alveoli into the lymphatics, which exist in large numbers in the interlobular tissue. Between the acini of a lobule there is much less connective tissue than between neighbouring lobules. Between the alveolar ducts and infundibula of the same acinus the quantity of connective tissue is extremely minute, while the walls of adjacent alveoli coalesce, so as to form only a single membrane.

IV.—*Blood-vessels.*

The larger bronchi are accompanied by branches of the **pulmonary artery** and vein. The artery and the bronchi branch together, and continue in company until the latter enters the acinus. Before this point is reached the vein separates from the artery and bronchus. Arrived at the acinus, the artery divides into a number of smaller branches, which resolve themselves into an extremely close plexus of capillary vessels, by which the walls of the alveoli are covered.

The **capillaries** project into the lumen of the air-spaces, and running a tortuous course, wind backwards and forwards through the alveolar walls, so as to project sometimes into one alveolus, sometimes into the other. They are covered by the large thin epithelial cells, while the small granular ones lie in the meshes of the vascular network. The artery, like the bronchus, enters the acinus at its apex, while the **veins** originate at the periphery of the acinus; and it is not until they have attained some size that they come to join the arteries and bronchi.

The walls of the bronchi down to their terminations in the acini are supplied by the **bronchial arteries,** and the blood is conveyed away by the **bronchial veins.** These vessels, of course, run with the bronchi. At the termination of the bronchi there is a more or less free anastomosis between the capillaries of the bronchial and those of the pulmonary systems.

V.—*Lymphatics.*

The lymphatics of the lung are very numerous. Their arrangement has been exhaustively studied by Klein. He describes three systems—(1), the **subpleural plexus,** lying in the pleura, and arising partly in this tissue, partly from the walls of the superficial alveoli. Some of the vessels from this plexus pass to the bronchial glands without entering the lung; but the most of them pass into the latter, and join (2), the **perivascular lymphatics,** which arise in the walls of the alveoli, and accompany the vessels as a plexus, or as sinuses. (3). The **peribronchial lymphatics** belong to the walls of the bronchi, with which they run, communicating frequently with the perivascular plexus. In the walls of the alveoli there exists under the epithelium a system of juice-canals, or a lymph canalicular system, which communicates with the interior of the air-spaces by stomata between the epithelial cells. Particles can readily pass from the cavity of the alveoli into the juice-canals, and the alternate contraction and expansion of the lung serve to force on the stream of lymph towards the bronchial glands, to which all the lymphatics converge. This passage of solid particles from the air-spaces into the lymphatics has been amply proved by experiments on animals, and can be seen in the body of almost every adult human being. The black pigment in the interlobular lymphatics and bronchial glands consist mainly of little masses of smoke, dust, &c., which have been introduced into the body with the inspired air. The cavity of the pleura communicates by true stomata between the endothelium on the surface of the lung with the juice-canals of the pleural connective tissue. There are stomata in the parietal pleura also, but they are absent on those parts which correspond to the ribs.

Along the course of the larger and smaller bronchi are accumulations of lymph corpuscles, sometimes grouped into follicles, at others more diffusely arranged. They are sometimes true lymph follicles; but sometimes the corpuscles lie merely in the ordinary connective tissue, and a reticulum, such as occurs in genuine adenoid tissue, is absent. Little masses of adenoid tissue are found frequently immediately under the pulmonary pleura (J. Arnold).

VI.—*Nerves*.

The nerves of the lungs are numerous, and consist of bundles both of medullated and of non-medullated fibres. They accompany the bronchi. Other terminations than those in the muscular tissue of the vessels and bronchi are unknown. Ganglia occur on the course of the nerve trunks.

1. The **trachea** of a child is hardened in chromic acid for a week or ten days, and then in alcohol. Transverse and longitudinal sections are made, stained in cochineal, and mounted in balsam.

Transverse Section.—The crescentic-shaped piece of hyaline cartilage is seen enclosed in fibrous tissue. The trachealis muscle completes the tube posteriorly. In man the muscular tissue is attached mostly to the extremities of the cartilage; but in the dog and the cat the attachment is to the outer or convex side of the cartilage at some distance from its extremities (Stirling). The submucous coat contains fat in places and the acini of the mucous glands. These appear as groups of roundish bodies. In some of these the epithelium is granular, in others clear and transparent. Klein thinks that the acini whose epithelium is granular were at rest, while those whose cells are transparent were secreting at the time of death. Crescentic-shaped groups of granular cells are often seen at the periphery of the supposed active acini. The ducts of the glands are lined by columnar epithelium, which is ciliated near their opening on the surface of the trachea. The mucous membrane contains a great deal of elastic tissue longitudinally disposed, consequently here cut across, and appearing as shining unstained points. In the mucous membrane are numerous lymph corpuscles, which are rarely grouped to form follicles. Numerous blood-vessels are seen in the mucous and submucous coats.

Under the epithelium is a very thick, homogeneous, basement membrane. This is much less distinct in lower animals. The epithelium is columnar ciliated. In good sections the ciliated cells, goblet cells, and replacement cells can be distinctly seen.

Longitudinal Section.—The pieces of cartilage appear of an oval shape, flatter on their outer than on their inner side. They are surrounded and joined together by fibrous connective tissue. Inside this is the submucous coat, with the glands, which are less numerous opposite to the pieces of cartilage than between them. In the mucous coat the elastic tissue will be seen to form a network, with long meshes. The basement membrane and epithelium appear as in the transverse section.

Ganglia are not so commonly seen in the human trachea as in that of the dog.

2. The **lungs** of a recently-killed cat are filled from the trachea with one-fourth per cent. chromic acid. The trachea is tied, and the distended lungs are placed in a large quantity of the chromic acid solution. In a week or ten days the lungs, now set in the distended condition, are cut into small pieces, washed in water until free of the chromic acid, and preserved in alcohol, which during the first few days must be changed two or three times.

Sections are best made with the freezing microtome, which for cutting the lungs is invaluable.

Sections should be made across the line of the bronchi, and others perpendicularly through the pleura.

A cross section of a middle-sized bronchus will show a fibrous coat enclosing oval or crescentic-shaped masses of cartilage. Inside this there is a continuous layer of circular muscular fibres: more internally, the mucous membrane, which contains many elastic fibres, here seen in transverse section. The lumen of the tube is stellate; this is due to the numerous longitudinal ridges in the mucous membrane, which are here cut across. The epithelium is columnar ciliated, and rests on a basement membrane, much thinner than that in the trachea. Mucous glands lie in the outer fibrous coat, and their ducts penetrate the other tissues. Accompanying the bronchus there is a branch of the pulmonary artery and vein. Bronchial arteries, bundles of nerve fibres, and possibly ganglia, may be seen. The lymphatics may be recognised as splits in the connective tissue, lined by spindle-shaped endothelial cells. Accumulations of lymph corpuscles (lymphoid follicles) are very common. All these structures are enclosed in a considerable quantity of loose connective tissue.

The pulmonary vein runs with the larger bronchial tubes, but in the more peripheral parts of the lung the veins run an isolated course.

The small bronchial tubes, still accompanied by the arteries, have a circular lumen, bordered by low cubical cells, scanty muscular tissue, no cartilage or glands.

Between the large vessels and tubes is seen the spongy tissue of the lung, consisting of the alveolar passages, and infundibula, with their alveoli, cut in various directions. A number of spaces are seen, some of which are small—these are the alveoli; others are larger—these are the infundibula and alveolar passages. If the latter are cut across between the openings of alveoli, the section is even and round; but if the section passes through the openings of the alveoli, then the outlines are irregular and scalloped, the depressions being the alveoli opening off from the central passage. The walls of some of the alveoli are seen in section; in others a surface view is got. On the latter numerous nuclei are seen. Some of these belong to the epithelium of the alveoli, but most of them are the nuclei of the capillary vessels.

By a fortunate chance the opening of a terminal bronchus into the alveolar passages may be seen, but this is not likely to occur unless a large number of sections are examined.

In sections made perpendicularly through the surface of the lung the pleura is seen running smoothly, while the sub-pleural tissue, of looser structure, dips into the lung between the lobules. The conical shape of the superficial infundibula may be seen.

3. Sections of the lung of a rabbit or cat whose air-spaces have been filled by a solution of nitrate of silver, one-half per cent., and which has been hardened in the distended condition by alcohol. Sections made by the freezing microtome, stained in logwood, mounted in glycerine. The outlines of the epithelial cells on the walls of the alveoli and infundibula are marked out by fine black silver lines, as in an endothelium. Some of these cells are large and unstained, resembling ordinary endothelial cells; others are smaller and granular, and stain brown with silver. The nuclei may be seen in many of the cells.

4. The lung is moderately distended with air, and the trachea tied. The blood-vessels are then injected with Prussian blue from the pulmonary artery, and the still inflated lung placed in alcohol. Next day it may be cut in pieces and the alcohol changed. Sections by freezing. Cochineal or picrocarmine; balsam. The close plexus of capillaries is seen on those alveoli which present themselves in face, and in the meshes are seen the red nuclei of the epithelium. When the alveolar walls appear in section, it can be seen how the capillaries project into the lumen of the alveoli, and twine backwards and forwards, so as to present themselves sometimes in one, sometimes in the other alveolus.

It will be noticed that a single artery gives capillaries for several alveoli, and that the arteries and veins lie at opposite sides of the capillary plexus.

CHAPTER XIII.

URINARY ORGANS.

I.—*The Kidney.*

The kidney is a compound tubular gland. All the tubes which enter into its composition run a similar course, which is very long, and complicated by numerous bendings and changes of structure. Before describing the tubes, it is necessary to call attention to certain divisions of the substance of the kidney and certain markings on it which can be recognised with the naked eye.

If a kidney be divided longitudinally into two equal parts by an incision passing through its convex edge, it will be seen to be a hollow organ, enclosing a cavity in its interior. This cavity is called the **pelvis**, and passes internally into the ureter, while on all other sides it is bounded by the kidney substance. This latter projects into the pelvis in the form of conical processes, which are called **papillæ**. In the kidneys of many of the lower animals there is only one papilla. In the human kidney there are several. Each papilla is the apex of a cone, the base of which is placed peripherally, but does not reach the surface of the kidney. The cone is called a **pyramid of Malpighi,** or simply a pyramid; and the sum of the pyramids form what is called the **medullary portion** of the kidney.

The pyramid has in all parts a streaky appearance, the striæ all diverging from the papilla. Next this, for about two-thirds of the entire depth of the pyramid, the striation is obscurely marked, but in the outer third it is very distinct, and in kidneys whose blood-vessels still contain blood it is seen to consist of alternate colourless and red lines—the former being urinary tubes, all running parallel to one another; the latter being blood-vessels, also running a parallel course. This outer part of the pyramid is distinguished from the inner by many important details of structure, and is called the **intermediate portion** or **border layer**, while the inner part is called the **papillary portion**. At the base of the pyramid sections of several large blood-vessels may be seen.

Covering the bases of the pyramids, and extending in between them, if there is more than one, is the **cortical portion** of the kidney, which extends to the surface of the organ. The structure of this is manifestly different from that in the pyramids. It still presents a radial striation. The lines of parallel tubes in the intermediate layer pass continuously into the cortex, and, becoming narrower as they extend towards the periphery, disappear a short distance below the surface. These extensions into the cortex are called the **medullary rays**, or **pyramids of Ferrein.** Between these are no longer parallel sets of blood-vessels, but a substance which looks granular, and which will be shown to consist of tubes twisted very much, and pursuing a most tortuous course, and consequently cut in all directions. This is called the **labyrinth.** In the labyrinth may be seen, in congested kidneys, minute red points. These are the **Malpighian bodies.** Those parts of the cortex of the kidney which dip down between the bases of the pyramids are called the **columnæ Bertini.**

Course of the Tubes.—The best description of the course of the urinary tubules is that which has been given by Klein. The following account is substantially the same as his, some of the less important subdivisions being omitted:—

Each renal tube commences in the labyrinth of the cortex of the kidney by a spherical or oval dilatation, called the **capsule of Bowman.** This opens by a constricted **neck** into a wide tube **(primary convoluted tube),** which is very much twisted, and lies in the labyrinth. After running for some distance this convoluted course, it enters a medullary ray, and, becoming straight or slightly spiral, passes to the junction of the cortex and medulla. Where it is situated in the medullary ray, it is called the **spiral tube.** Arrived at the base of the pyramid, it suddenly diminishes in diameter, and the narrowed tube, which is known as the **descending limb of Henle's loop,** runs straight through the intermediate portion and into the papillary portion of the medulla, where it abruptly bends on itself, and runs back again through the intermediate portion and into the cortex. The bend is called **Henle's loop.** Shortly after making the bend, the tube becomes wider, and is called the **ascending limb of Henle's loop.** This, when it reaches the cortex, enters a medullary ray, and, with numerous changes of diameter, runs for some distance in this towards the surface of the kidney. Sooner or later it leaves the medullary ray, and, entering the labyrinth, it presents a number of sharp bends and sudden variations of calibre, so as to justify the term **irregular tube** applied to this portion. The irregular part of the tube, after a short course, changes its character, and passes into a portion which in every respect resembles the primary convoluted tube, and which is called the **secondary convoluted or intercalated tube**, and which lies in the labyrinth. The intercalated tube is continuous with a narrow tube **(arched portion of excretory tube)** which pursues an arched course, with the convexity turned towards the surface of the kidney, and which opens into a tube which, running in a medullary ray, passes straight into the pyramid, and through the two portions of this to the apex of the papilla, where it opens into the pelvis. This **straight excretory tube** receives in the cortex great numbers of the narrow arching tubes, each of which is the termination of a tube which has originated in a Bowman's capsule and pursued the course which has been described. The straight tube also joins with other straight tubes, chiefly in the pyramid; and as these tubes coalesce, the calibre of the resulting tube increases. Close to the apex of the papilla a great number of straight tubes join, and the resulting tubes, of which in the human kidney there are from ten to twenty-five opening on each papilla, are called the **papillary ducts.**

To recapitulate, we have in succession—1. Bowman's capsule, opening by a constricted neck into 2. primary convoluted tube; 3. spiral tube; 4. descending limb of Henle's loop; 5. ascending limb of Henle's loop; 6. irregular tube; 7. intercalated tube; 8. narrow arched portion of excretory tube; 9. straight excretory tube; 10. papillary duct.

The part of the kidney where each of these is found is given in the following table:—

Cortex,	Labyrinth,	Bowman's capsule.
		Primary convoluted tube.
		Irregular tube.
		Intercalated tube.
		Arched portion of excretory tube.
	Medullary ray	Spiral tube.
		Ascending limb of Henle's loop.
		Straight excretory tube.
Medulla,	Intermediate portion,	Descending limb of Henle's loop.
		Ascending limb of Henle's loop.
		Straight excretory tube.
	Papillary portion,	Bend of Henle's loop.*
		Straight excretory tube.
		Papillary duct.

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* The bend of Henle's loop is sometimes found in the intermediate portion of the pyramid, and the structure of the tube at the bend sometimes resembles that of the *ascending* limb of the loop.

The total average length of each uriniferous tube in the human kidney has been calculated to equal 50 mm., or about two inches.

It is evident that, owing to the coalescence of the excretory tubes, one papillary duct is in connexion with a very large number, probably about one thousand capsules of Bowman. Each duct may be considered the place of coalescence of a system of tubes whose collecting and spiral tubes lie in one medullary ray, and whose convoluted tubes and Bowman's capsules lie in the circumjacent labyrinth. Such a portion of renal substance may be looked on as an unity, as has been done by Ludwig, who speaks of it as a **primitive cone or lobule**.

Structure of the Tubes.—Near the apex of the papillæ the tubes are separated by a considerable quantity of connective tissue, and here they have, besides their epithelium, no proper wall separable from the surrounding tissue. In every other part of their course they are formed by a **homogeneous basement membrane,** containing at intervals a few nuclei, and lined by a single layer of **epithelial cells.** These cells differ considerably in the different portions of the tube.

The interior of Bowman's capsule is lined by flat cells. At the neck the cells become cubical. In some lower animals the portion of the tube corresponding to the neck is very long, and lined by ciliated epithelium. In mammals the neck is much shorter, and, although cilia have been described as occurring in this part of the tube in the sheep, horse, rabbit (Hassall), and mouse (Klein), it cannot be said as yet that their presence is general. In the convoluted tubes the cells are large, polygonal bodies. Each cell has a rounded nucleus situated about its middle. External to the nucleus it presents a radial striation similar to what was seen in the epithelium of the salivary tubes, and which seems to be due to a number of rods, or very fine tubes, embedded in the protoplasm of the cell. The spiral tube has the same kind of epithelium. The descending limb and the bend of Henle's loop have flat epithelium. The cells are thickened in the middle, where the nucleus is placed, and appear spindle-shaped in profile view. As soon as the ascending limb of the loop re-enters the intermediate portion it widens, and regains an epithelium, which contains rods, but, corresponding to the less size of the tube, which in some of its narrow parts is very thin indeed, the cells are of less height than in the primary convoluted and spiral tubes. In the irregular portion of the tube the rod structure of the epithelium is much coarser than in any other part of the whole tube. The nuclei lie very near the inner ends of the cells, and, owing to the sharp bends in the tubes, the rods are frequently disposed obliquely to the wall. In the intercalated portion the epithelium is similar to that in the primary convoluted tube; and here the rod epithelium ceases. The arched excretory tubes have flat or cubical, and the straight excretory tubes and the papillary duct have columnar epithelium, showing no vestige of rods, and becoming progressively higher as the lumen of the tube increases. The following Table gives the kind of epithelium found in each part of the tube:—

Flat epithelium, . .	Bowman's capsule. Descending limb and bend of Henle's loop. Arched excretory tubes.
Striated, or rod epithelium, . . .	Primary convoluted and spiral tubes. Ascending limb of Henle's loop. Irregular tube. Intercalated tube.
Columnar epithelium, without rods. . .	Straight excretory tube. Papillary duct.

The path of the blood through the kidney equals in complexity the arrangement of the tubules. The large divisions of the renal **artery,** after giving off some branches to the mucous membrane of the pelvis, pass to the bases of the pyramids, where they divide into large vessels, which form **arches** between the cortical and medullary portions. These vessels do not communicate with one another, so that the arches are not complete. From the arches there come off large numbers of vessels, which run radially through the cortex (always in the labyrinth) to near the surface of the kidney. These are the **straight ascending,** or **interlobular arteries.** From all sides of these there come off minute arteries, each of which bends slightly backwards towards the medulla, and then perforates* the membrane of one of Bowman's capsules at a point nearly opposite to that at which the convoluted tube emerges. This small artery is the **vas afferens.** Having entered the capsule, by a rapid process of division, it breaks up into twenty to thirty capillary vessels (the **glomerulus**), which form loops, whose convexity is directed away from the point of entrance of the vas afferens, and which do not communicate with one another. The capillaries converge to a small vein **(vas efferens),** which leaves the capsule close to the point of entrance of the artery, and then breaks up again into **capillary vessels,** which surround the tubules with a close plexus, whose meshes are polygonal in the labyrinth, more elongated in the medullary rays.

Prior to entering the capsule of Bowman the vas afferens frequently gives off a minute twig, which passes direct to the tubular capillaries, without having taken part in the formation of the glomerulus.

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* This is not strictly true. The glomerulus really lies external to the wall of the uriniferous tube, and receives a covering from it. The glomerulus pushes this before it into the interior of the capsule. The whole is somewhat analogous to a serous membrane. The covering of the glomerulus corresponds to the visceral layer, the outer wall of Bowman's capsule to the parietal layer, and the slit-like interspace to the serous cavity.

The interlobular arteries sometimes terminate by dividing into their last vasa afferentia, but more frequently they extend to the surface of the kidney, and having given off some twigs to the most superficial tubules, terminate by anastomosing with the vessels of the capsule.

The **veins of the cortex** arise by coalescence of the tubular capillaries. On the surface of the kidney several small veins coalesce, and form stellate figures, known as the **stellulæ of Verheynius.** From these are formed veins **(interlobular veins)**, which pass through the cortex in company with the interlobular arteries, receiving on all sides smaller vessels derived from the tubular capillary plexus. At the junction of the cortex and medulla the interlobular veins pass into **venous arches,** which run at the bases of the pyramids, and which anastomose with one another, to form the great tributaries of the renal veins.

The medullary portion of the kidney is supplied by vessels which run in the interspaces between the medullary rays. These vessels are called **vasa recta.** They arise in different ways. Some few are true arteries, and come off from the arterial arches, which give origin to the interlobular arteries of the cortex. Others are the vasa efferentia of the innermost Malpighian glomeruli; and again, others are veins originating from the tubular capillaries of the cortex. Whatever their origin, they divide into a number of branches, which run in bundles parallel to one another. They pass into capillaries, which supply the tubes of the pyramids, and, bending round, coalesce to form straight veins, which pass back through the same fissure in the intermediate portion as those in which the arteries lie, and finally pass into the venous arches at the bases of the pyramids. A special network of small veins exists about the papillary ducts. It communicates with the other vessels of the pyramid.

The vessels which form the glomerulus do not hang naked into the interior of the capsule, but they are covered by a layer of epithelium, whose cells are flattened, and which is continuous with that lining the interior of the capsule. Between the capillaries is a small quantity of connective tissue. This epithelium and connective tissue are important, as it is in them that the earliest changes occur in some, if not most, forms of Bright's disease.

There is a layer of the cortex next the periphery which contains no glomeruli or capsules, and another thinner layer next the pyramids equally free from these structures.

The glomeruli are not all of the same size. In animals those near the periphery are smaller than those more deeply situated, while in men large and small glomeruli are irregularly distributed in the cortex.

There is a plexus of **lymphatics** in the capsule of the kidney, and another in the connective tissue which accompanies the larger vessels. These plexuses communicate with one another. Besides these, lymphatic spaces have been described between the tubules.

The **nerves** are mostly of the non-medullated kind, and run with the arteries. They have some microscopic ganglia on their course. Their terminations are unknown.

The kidney is surrounded by a mass of adipose tissue. Inside this is the **proper capsule,** consisting of fibrous and elastic tissue and some smooth muscular fibres. The deeper part of the capsule is of loose structure, and is continuous with the interstitial connective tissue. This exists in most places in very small quantity, but in the papillæ there is a considerable amount of dense connective tissue. Also surrounding the larger vessels and around each of Bowman's capsules more or less connective tissue is always found.

Twigs from neighbouring arteries—suprarenal, lumbar, phrenic—reach the surface of the kidneys, and there anastomose with the most peripheral branches of the renal vessels.

The **pelvis of the kidney** is lined by transitional epithelium. This rests on the mucous membrane, formed of fibrous and elastic tissue, and which has no papillæ, and is said to possess a few acinose mucous glands and lymphatic follicles. The submucosa contains fat. Outside this is smooth muscular tissue, arranged in two layers—an inner radial, an outer circular—and continuous with the muscular coat of the ureter. On the renal papillæ the circular layer of fibres is most developed. The outer layer of the capsule of the kidney is continuous with the connective tissue which ensheaths the renal vessels, while the deeper loose layer extends into the hilum as far as the attachment of the mucous membrane of the pelvis.

II.—*The Ureter and Bladder.*

The **ureter** is lined by transitional epithelium. Acinose glands have been described in the mucous membrane, but their existence is doubtful. The muscular coat consists, in the upper part, of an external circular and an internal longitudinal layer of fibres. To these there is added in the lower part, a third, external layer of longitudinal fibres. There is a nervous plexus in the muscular coat.

The epithelium of **the bladder** is transitional, but when the organ is distended all the cells are flat. There are numerous acinose glands in the mucous membrane of the bladder.

The muscular coat is composed exclusively of unstriped fibres, lying in several badly differentiated layers. In the outer layer longitudinal bundles of fibres preponderate. In the inner layer the bundles have a more circular course.

The bladder is abundantly supplied with nerves, on which are seated numerous ganglia.

The kidney should be divided longitudinally or transversely, placed for two days in five per cent. neutral chromate of ammonia, washed in water, and then placed in alcohol, which should be changed once or twice, until the hardening is completed. The sections should be as large as possible, and it is essential that they should be made either radially from the papilla to the surface, or at right angles to this—that is, tangentially.

1. **Radial Section.**—Stained in logwood or cochineal; mounted in balsam, or, in order to see fine details of structure, in glycerine or Farrant's solution. With a low power observe in the cortex the alternate lines of straight tubes (medullary rays) and convoluted tubes (labyrinth). Observe in the latter the Malpighian bodies lying in two rows, and notice their absence from a zone extending for some distance from the surface of the kidney.

Trace the medullary rays into the intermediate zone, and observe here the tubes, mostly dark and distinct, and all running parallel to one another. The apparently granular tubes are the ascending limbs of Henle's loops. These cease abruptly at the junction of the intermediate and papillary portions. In the latter the tubes are much less opaque, and become very large as the papilla is approached. Sections of large arteries and veins are seen at the junction of the cortex and pyramid.

With the high power try to make out the different portions of the tube. This is not difficult, if a diagram be drawn of the entire tube, so as to show in what parts of the section its different portions lie.

a. In the **labyrinth** observe the Malpighian bodies. The loops of capillaries will be seen in the interior of Bowman's capsule. This latter is lined internally by very thin, flat cells, appearing spindle-shaped on section. The tuft of vessels presents a great number of nuclei, most of which belong to the capillaries, but some to the epithelium and connective tissue. The afferent and efferent vessels may be seen, and in a fortunate section the opening into the convoluted tube. From some of Bowman's capsules the glomeruli may have fallen out, leaving round holes. Each capsule is surrounded by some connective tissue. All about the Malpighian bodies are sections of the primary convoluted tubes, cut in various directions. Choose a cross section. Observe the basement membrane; the high epithelium leaving a very small lumen: the round nuclei and the striation of the outer parts of the cells. Choose a long section. Focus for the upper surface. Observe the coarsely granular appearance of the cells. The apparent granules are the ends of the rods. Find an irregular tube. This is readily recognised by its irregular contour, its strongly-marked rod epithelium, and its nuclei, which stain very deeply, and lie near the inner ends of the cells. The intercalated tube is not to be distinguished from the primary convoluted. The arched excretory tube is recognised by its small size, its coalescence with other tubes, and its epithelium without rods. Running radially through the labyrinth between the rows of Malpighian bodies are the interlobular arteries and veins, which do not differ from similar objects in other parts. The arteries will be recognised by their transverse muscular coat, and the thin-walled veins often contain blood.

b. In the **medullary ray** all the tubes are cut in length, and are seen to run parallel with one another. The spiral tube, in structure identical with the primary convoluted, forms the most prominent constituent. The ascending limbs of Henle's loops are much narrower, in places extremely narrow, but still with opaque rod epithelium. The straight excretory tubes fewer than the others, but easily distinguished by their smooth transparent epithelium and relatively large lumen.

c. **Intermediate portion.**—Here the most conspicuous objects are the ascending limbs of Henle's loops. They are wider here than in the cortex. The straight excretory tubes are as in *b.* The descending limbs of Henle's loops are thin tubes, resembling capillary blood-vessels. They are distinguished by having a greater number of nuclei, and having a basement membrane outside the epithelium, by not branching or anastomosing, and by their connexion with either the termination of a spiral tube or with the commencement of an ascending limb of the loop. At the base of the pyramid sets of vasa recta are seen to alternate with sets of straight tubes.

d. **Papillary portion.**—The tissue here is much more transparent than in the intermediate portion, owing to the absence of tubes with rod epithelium, which is very opaque. The loops of Henle may be seen; they generally occur near the intermediate zone, but some reach pretty far down into the papillary part. They must not be confounded with looping capillary blood-vessels. The straight excretory tubes and papillary ducts form, with the blood-vessels and connective tissue, the remainder of this part.

2. **Tangential Sections**—*a.* Through **cortex.**—The labyrinth will be seen to be a continuous structure, through which pass at intervals sets of straight tubes (medullary rays), running radially, and here cut across. The cross view of the tubes of the medullary ray will complete the notions of their structure, got by seeing them in length in the radial section.

b. Through **Intermediate portion.**—All the tubes cut across. The ascending limbs of Henle's loops recognised by their coarse rod epithelium, the descending limbs appearing as thin tubes, lined by flat epithelium, the central portion of each cell corresponding to the nucleus bulging into the lumen of the tube. The excretory tubes with relatively large lumen, and low columnar epithelium without rod structure. Cross sections of sets of vasa recta are seen among the tubes.

c. Through **Papillary portion.**—Excretory tubes much larger. Lumen large. Epithelium distinctly columnar. Papillary ducts are wide tubes, with beautifully regular columnar epithelium. The tubes are separated by a considerable quantity of dense connective tissue.

3. **Injected Kidney.**—Radial section of kidney whose blood-vessels have been injected with Prussian blue; cochineal; balsam.

The distribution of the vessels will be readily understood from the description already given. The formation of the Malpighian glomerulus will be much more distinct than in uninjected preparations.

4. Small bits of fresh kidney placed in a mixture of strong hydrochloric acid one, water three parts, for twenty-four hours, and then in water for twelve hours. The connective tissue is dissolved, and considerable lengths of the tubes can be isolated. It is by this method, rather than by sections, that the course of the tubes has been made out. The acid injures the epithelium, but not to such a degree as to render the different parts of the tube indistinguishable from one another. The tubes are very brittle. It is better to place small fragments of the softened kidney in a drop of water on the slide, and try to shake them asunder by tapping the glass, rather than to attempt to separate the tubes with the needle. The examination is best made with a low power without a cover-glass. If this latter be applied, it should be supported at the edges by small fragments of thin glass, so that it may not press on the object. Bowman's capsules, attached to considerable lengths of the convoluted tubes, are commonly seen. The abrupt passage of the spiral tube into the thin descending leg of Henle's loop, complete loops, and the coalescences of excretory tubes, are all frequently met with. If the kidney of a small animal, such as a mouse, be used, very considerable portions of the whole length of a tube may be got, as, for example, the whole length from Bowman's capsule to the descending limb of Henle's loop, inclusive; or the two limbs of the loop, the irregular, intercalated and commencement of the excretory system. By piecing these different fragments together, the entire course of the tube may be made out.

5. Small fragments of the cortical portion of the kidney, preferably of a rat, macerated twenty-four hours in five per cent. neutral chromate of ammonia, torn up, and examined in a drop of glycerine, show the separated epithelial cells of the convoluted tubes. The rods to which the radial striation is due are seen embedded in the protoplasm of the cells. Some of the cells may be broken and the rods isolated, showing that they are not mere markings on the cells.

6. The transitional epithelium of the excretory urinary passages has been already examined. To see it *in situ*, the bladder

of a dog should be moderately distended with 0.25 per cent. chromic acid, and placed in the same fluid. After a week it should be washed, and the hardening completed in alcohol. Vertical sections, stained in logwood or picrocarmine, are mounted in glycerine.

The ureter may be similarly treated. It is to be noted that in the upper part of the ureter, contrary to the usual arrangement, the circular muscular coat is external, the longitudinal internal.

CHAPTER XIV.

MALE ORGANS OF GENERATION.

I.—*Testicle.*

THE testicle is a compound tubular gland. It is covered by a serous membrane, **tunica vaginalis,** under which is a dense fibrous capsule, the **tunica albuginea**. At the posterior edge of the testicle the capsule becomes continuous with an elongated mass, composed of fibrous tissue, mixed with smooth muscular fibres, and which projects into the gland almost its entire length. This is the **mediastinum testis**, or **corpus Highmori.** From the anterior and lateral surfaces of the mediastinum, **septa** extend to the inner surface of the tunica albuginea, and imperfectly divide the organ into a number of somewhat conical-shaped compartments or lobes. In many animals the corpus Highmori lies, not at the surface of the testicle, but in the centre, and septa pass off from it on all sides.

In the compartments bounded by the septa testis lie the **convoluted tubes,** which secrete the semen. These are very numerous, and each tube is of great length. At their commencement they anastomose with one another and form loops, and they possess short diverticula which end blindly, and others which join with corresponding diverticula of neighbouring tubes. The convoluted tubes retain the same diameter throughout their course, but by coalescence with one another they diminish in number as they approach the apices of the conical compartments in which they lie. Finally, each tube suddenly narrows, and passes into a straight canal, which enters the corpus Highmori. In the human testicle there are from one hundred to two hundred of these straight tubes, or **vasa recta,** while there may be as many as nine hundred convoluted tubes. In the mediastinum the vasa recta become somewhat dilated, and break up into a close network, the **rete testis,** from which there come off ten to twenty vessels **(vasa efferentia)**, which perforate the mediastinum at its upper part. At first each of these vessels runs a straight course, but it soon becomes very tortuous, and forms a convoluted mass of a conical shape. Hence these vessels are called **coni vasculosi.** From the uppermost of these commences the **epididymis,** a tube of enormous length and tortuosity, and which forms a convoluted, elongated mass, extending along the posterior part of the testicle. At its upper part it receives in succession the coni vasculosi. At its lower part it becomes less convoluted, and passes into the **vas deferens**, which, bending round at an acute angle, passes again upwards, and conveys the semen to the urethra. Near the point where the epididymis passes into the vas deferens, it gives off a diverticulum, often of considerable length, which passes upwards and ends blindly. This is the **vas aberrans.**

The structure of each part of this complicated tube has now to be described.

1. **The Convoluted Tubes.**—The wall is formed internally of a thin homogeneous basement membrane. Outside this is a laminated tissue, which consists of delicate membranes, covered by flat endothelioid cells. The laminæ lie in several layers, one over the other, but each of them is perforated by holes, so that the spaces between them communicate freely. The tissue composed of these laminæ passes insensibly into that which lies between the tubes, and which will be subsequently described. The thickness of the wall of the tubes is greater in large animals than in small, and is particularly great in man.

In the interior of the tubes is a mass of epithelial cells, lying in more or less well-defined layers over one another, and nearly filling the lumen. It will be seen immediately that these cells are not all separate from one another, but really form, in many instances, protoplasmic masses, each of which contains several nuclei. As the masses tend to assume a radial position in the tube, they may extend through the different layers, and may contain nuclei belonging to each of these. In sections it is not always easy to see how far the protoplasm is divided: this is best made out when portions of the tubular contents are torn up. Most externally are cells whose nuclei are small, but stain very deeply. In suitably prepared objects it can be seen that these nuclei have no proper membrane, and that they consist of stained threads, forming figures generally somewhat stellate, with less deeply-stained substance between. In fact, they present the appearances seen in nuclei which are undergoing karyokinetic division. Lying at intervals among these cells are others, which have larger nuclei, generally oval in shape, with a well-marked bounding membrane and nucleoli. These nuclei stain only feebly, and are in a quiescent or resting condition. Internal to this first layer are two or three rows of cells, possessing nuclei which are much larger than those of the outer layers. These nuclei also present evidences of active karyokinesis. But the threads here form usually a network or convolution, and there is a much larger quantity of unstained material between the stained threads than there was in the nuclei of the cells next the wall of the tube.

Next the lumen of the tube is a mass of cells, whose nuclei are well defined, small, round or oval, and which stain very feebly, and do not show any signs of undergoing division.

Lying at intervals among these inner cells, and sometimes extending between those of the second layer, are groups of elongated, deeply-stained bodies, arranged like the barbs on a feather. These are the heads of the spermatozoa. From them long thread-like prolongations, the tails, can frequently be seen extending into the central portion of the tube. The spermatozoa forming each group are not free, but are embedded in a peculiar structure, which reaches to the wall of the seminal tube. This body may be called a **spermatoblast,** although this term has been applied by many writers to other structures. The spermatoblast rests by an expanded foot on the wall of the seminal tube, and in this expansion is contained one of the oval resting nuclei described as occurring in the outermost layer of cells. From this the spermatoblast projects towards the centre of the tube as a columnar body, whose sides are marked by the impressions of the cells which lie about it. It sometimes contains a second nucleus in this part of its course, and it sometimes happens that there is no nucleus where it is attached to the wall of the tube, but that the nucleus is situated higher up. As the spermatoblast approaches the central portion of the tube it expands, and its expanded head becomes deeply lobed. In each lobe there lies, according to the stage of development of the spermatoblast, either an oval nucleus similar to those of the third layer, a completely-formed spermatozoon head, or an intermediate stage between these. When the development is in an advanced stage, there projects from each lobe a long filament or tail. Enormous masses of these filaments sometimes occupy the axial portions of the tube, where they are twisted into a sort of vortex. The spermatoblasts are very large and conspicuous objects in the testicle of the rat, where nothing is easier than to see them, and to trace their connexion with the wall of the tube. In other animals they are much thinner, and in sections it is often difficult to trace the connexion between the foot and the head, with its nuclei, although in carefully torn preparations this can readily be done.

It is by no means easy to explain the connexion which exists among the elements which form the contents of the seminal tubes. Very different views have been put forward by different writers, and the subject is as yet one which cannot be looked on as settled. The following explanation, however, seems best in accordance with the facts :—The appearances of the nuclei in the first two layers of cells are such as to make it almost certain that in them an active process of multiplication is in progress. The nuclei of the second layer are a later stage in the division than those in the first; while the nuclei of the third layer are the result of the division of those of the second, but themselves divide no further.

The protoplasm which surrounds these nuclei in all the layers is finely granular, and in sections the outlines marking the division of this into cells are often exceedingly faint and difficult to see. In torn preparations it can be made out that there is not a distinct and separate cell for each nucleus, but that tracts of undivided protoplasm exist, which contain in their interior several nuclei. Consequently, we may conclude that the nuclei multiply much more rapidly than the cells. Since the cells all grow in the confined space of the tube, where the only direction in which expansion can occur is towards the axis, the protoplasmic masses will naturally tend to assume a columnar shape. In such columnar masses, which contain several nuclei, one of these comes to rest—that is, it ceases to divide, and remains at the attached end of the protoplasmic column, and becomes the nucleus of the foot of the spermatoblast. The other nuclei come to lie at the opposite, or central end of the column (or, as it may now be called, spermatoblast), having assumed this position either by an active process of migration, or, more probably, by being pushed inwards by the surrounding masses of growing cells. Here, having terminated their divisions, and assumed the characters of the nuclei of the third layer, the protoplasm containing them becomes deeply notched, so that the inner end of the spermatoblast consists of a number of lobes, free at their inner ends, but all attached to a common stalk at the outer end, or that next the wall of the tube. Each lobe contains one nucleus. This becomes the head of the spermatozoon, while the tail is developed from the protoplasm of the lobe. The details of this development will be described subsequently. As the group of spermatozoa approach maturity, the quantity of protoplasm in the spermatoblast diminishes, and this becomes both shorter and thinner than before; so that when the spermatozoa finally become free, there remains only a small quantity of protoplasm about the basal resting nucleus. What becomes of this residue is uncertain. It may undergo atrophy, and disappear, or, after a period of rest, the nucleus may again become active, and by its division give rise to a new crop of spermatozoa.

Between the spermatoblasts, which lie at intervals, are masses of cells, with multiplying nuclei. When the spermatoblasts discharge their spermatozoa, these cells develop into a second generation of spermatoblasts, and carry on the process of spermagenesis.

Even in the most active testicle all the tubes are not engaged in secretion at the same time, but in different tubes, and different parts of the same tube, we find places where there are neither spermatoblasts nor spermatozoa, but simply protoplasmic masses with nuclei.

In immature animals, and commonly in men who die of disease, the tubes of the testicle contain cells whose nuclei present no evidence of division, but are all in the resting condition; that is, they have a distinct nuclear membrane and nucleoli.

In young animals the tubes contain some cells of very large size, each with a single large spherical nucleus. These cells have been compared to the ovarian ova. They are probably cells which, instead of multiplying by division, have merely increased in bulk.

A **spermatozoon** consists of a head, a middle piece, and a tail. The head varies very much in shape and size in different animals. In man it is an irregular flattened oval, narrower and thinner at its anterior end than where it is attached to the middle piece. In the rat it is hooked; in many birds pointed and twisted like a cork-screw; in frogs it is long and pointed, but straight.

The middle piece is of variable length, and is in man much narrower than the head, to the posterior end of which it is attached by a narrow neck.

From the middle piece comes off the tail, which is a long, very thin filament, by whose movements the locomotion of the spermatozoon is effected.

Attached to the middle piece and tail of the spermatozoon of the newt is a delicate membrane, whose free edge is thickened, and appears as an undulating fibre. A similar structure has been described in the spermatozoa of mammalian animals and of man (Gibbes), but these observations require confirmation.

The nuclei of the third layer, when they are about to change into the heads of the spermatozoa, elongate, and separate distinctly into two parts, one which is crescentic in shape, and stains deeply, and lies next the lumen of the tube; the other lies externally, is oval, and is embraced by the stained portion. This can be seen very readily in the testicle of the rabbit (Brissaud).

When the lobes of the spermatoblast are formed, the nuclei lie at first near their free ends, but subsequently pass downwards towards the attached end of the spermatoblast, where they undergo their further development. There can be very clearly seen, particularly in the rat, the head attached to the base of the spermatoblast by a fine thread of protoplasm, which is continued beyond the head, and which then swells out in the position previously occupied by the lobe of the spermatoblast. From this swollen piece the tail comes off. On the detached spermatozoa there can still be seen the protoplasmic swelling, corresponding to the position of the lobe, which is nearly that of the junction of the middle piece with the tail. This is the case, not only in the rat, but in the rabbit and other animals, and is particularly clear in the frog.

It is generally stated that the middle piece of the spermatozoon grows from the head. Even if this be so, there can be but little doubt that it receives, in addition, a covering from the protoplasm of the lobe of the spermatoblast, which also probably gives a sheath for the head. The tail is derived from the protoplasm of the spermatoblast.

Thus the spermatozoon consists of a head derived from the nucleus, a tail spun out from the protoplasm, and a middle piece whose origin is uncertain. It would appear that the whole nucleus does not become transformed into the head, but only that part which forms the intranuclear reticulum, and which, from its property of staining intensely in many dyes, is called chromatin (Flemming).

Filling up the spaces between the convoluted tubes of the testicle and the blood-vessels is **connective tissue,** which in most animals is mainly composed of delicate membranes, clothed with endothelial cells. This tissue is continuous with the walls of the seminal tubes and with the septa of the testicle. There occur also peculiar cells, which are usually arranged along by the walls of the blood-vessels, and lie in the meshes of the connective-tissue lamellæ. In some animals they constitute almost the entire interstitial tissue of the testicle. They are large, polygonal or irregular-shaped cells, coarsely granular, and very commonly contain pigment or fat. There is great uncertainty as to their real nature. There are very similar cells in the interstitial tissue of the ovary, and here their origin has been traced to the epithelium of the tubes of the Wolffian body (Balfour). A similar origin has been assumed by Klein for these cells of the testicle.

They are, however, considered by some to be of connective-tissue origin, and to be related to the so-called plasma cells.

2. The tubes of the **vasa recta** and **rete testis** are lined by a low cubical, almost flat, epithelium. There is a proper basement membrane in the vasa recta, but the tubes of the rete testis lie hollowed out in the fibrous tissue of the corpus Highmori.

3. The **coni vasculosi** and the **epididymis,** with the **vas aberrans,** have a high, columnar, ciliated epithelium. The cilia of each cell are commonly adherent to one another. These cells rest on a connective-tissue membrane, outside which is a thick circular muscular coat, with a connective-tissue adventitia outside all. The convolutions of the tubes are held together by loose connective tissue.

The **lymphatics** of the testicle commence in the interspaces of the lamellar interstitial tissue, which communicate with the spaces between the lamellæ of which the walls of the tubes are formed. These spaces open into vessels with proper walls, and which lie in the septa and tunica albuginea.

The **blood-vessels** ramify primarily on the inner aspect of the tunica albuginea and on the septa, forming what is called the tunica vasculosa: also in the corpus Highmori. From these parts capillaries come off, which form a close plexus around the tubes.

In the epididymis there is a very beautiful and close network of vessels lying in the mucous coat, close under the epithelium, an arrangement which has given rise to the view that this part of the seminal tube is not merely excretory, but that in it some important process of secretion takes place (Mihalcovics).

The course and terminations of the **nerves** of the testicle are entirely unknown.

The **vas deferens** is lined by columnar epithelium, which in the lower portion of the tube is ciliated. It rests on a mucous membrane, formed of connective and elastic tissue, and which is surrounded by a very thick muscular coat, consisting of an external longitudinal and an internal circular layer, to which in most parts is added an innermost longitudinal layer. Outside all is a connective-tissue adventitia, in which longitudinal bundles of smooth muscular fibres are contained. It is to the great thickness of its muscular coat that the vas deferens owes its hard cord-like feel. In the upper part of the vas deferens its mucous membrane presents numerous folds and diverticula, from the bottom of which open tubular glands.

The **vesiculæ seminales** may be looked on as an exaggerated form of these diverticula. Their mucous membrane, which is thrown into numerous folds, is lined by columnar epithelium. The muscular coat consists of three layers, of which the inner longitudinal is thickest.

The **ductus ejaculatorii** have a similar structure to the vas deferens, but their coats are thinner, and prior to their opening into the urethra the epithelium changes to a stratified transitional form.

II.—*Prostate.*

The **prostate** consists largely of bundles of smooth muscular fibres, which run among one another in various directions, separated by only a small quantity of connective tissue. Embedded in the fibro-muscular tissue is a large number of branching tubular glands, lined by columnar epithelium. There are two large ducts, which open one on each side of the colliculus seminalis, and in which coalesce a considerable number of the gland tubes. Several smaller ducts open further forward. The blood-vessels form a plexus about the gland tubes. Ganglia and Pacinian corpuscles are found in connexion with the nerves of the prostate.

In the interior of the glandular cavities of the prostate concentrically-laminated bodies are commonly seen, which are sometimes of microscopic size, but may form considerable concretions.

III.—*Cowper's Glands.*

Cowper's glands are acinose glands, whose ducts and acini are lined by columnar epithelium.

IV.—*Urethra.*

The epithelium which lines the **urethra** is in the upper part transitional; in the cavernous portion columnar; in the fossa navicularis compound squamous. The mucous membrane consists of fibrous and elastic tissue, and outside this is a muscular coat composed of circular and longitudinal fibres. Numerous branching tubular glands (glands of Littré) open into the urethra.

V.—*Penis.*

The skin of the **penis** is delicate, and, corresponding to the anterior part of the organ, has few or no hairs. On the inner aspect of the prepuce and around the base of the corona glandis are sebaceous glands. This is one of the few situations in which sebaceous glands exist independently of hairs.

The subcutaneous tissue contains no fat.

The **corpora cavernosa** are enclosed each in a dense fibrous capsule (tunica albuginea), which contains a great quantity of smooth muscular tissue. From the interior of the capsule septa of fibromuscular tissue pass in all directions, and anastomosing, enclose spaces which give to the tissue its peculiar spongy appearance. These spaces are lined by an endothelium, and are in direct continuity with the veins of which they form the dilated commencements. The arteries run in the septa, and divide into capillaries, which open into the cavernous spaces, from which the blood is carried off by the veins. During erection these spaces are enormously distended with blood.

The **corpus spongiosum urethræ** is similar in structure to the cavernous tissue of the penis, but there is less muscular tissue in the trabeculæ. There is also in the submucous tissue of the urethra a venous plexus, with greatly dilated vessels, and which is continued back to the membranous portion.

Pacinian corpuscles, which will be described in the chapter on the skin, are found on the nerves of the skin of the penis. On the glans the nerves terminate in peculiar end-organs, named **genital corpuscles.** These are bodies generally of an oval shape, and situated in the papillæ of the mucous membrane. Each of them contains in its axis a nerve-fibre, which loses its white substance on entering the corpuscle, and then runs as a naked axis cylinder, and terminates in a swollen extremity. It frequently branches, and then each branch ends in a little dilatation. Surrounding the nerve-fibre is a longitudinally-streaked substance, resembling the core of a Pacinian corpuscle. Outside this is a layer of tissue, which contains transversely-disposed nuclei, and the whole is enclosed in several connective-tissue laminæ, continuous with those of the perineurium of the nerve, and, like them, clothed on both surfaces with endothelial cells.

The **ejaculated semen** contains, besides the spermatozoa, fluid derived from Cowper's glands and the prostate; desquamated epithelium and spherical bodies, which stain deeply (seminal granules), but whose nature is uncertain; also occasionally prostatic concretions and a peculiar substance which, when the semen is dried, crystallises in long octahedral crystals, and which is the phosphatic salt of an organic base called spermatin. This is derived from the prostate.

1. **Testicle** of a rabbit or cat, hardened in Müller's fluid and alcohol. Section transversely through the whole testicle and epididymis. Logwood; balsam. The section is extremely fragile, and some difficulty will probably be experienced in getting it perfect on the slide. The best method is to float the section on to the slide from the alcohol, and, after removal of as much fluid as possible with filter-paper, to treat the section on the slide with oil of cloves.

With the low power the fibrous capsule and the septa converging to the centrally-placed corpus Highmori will be seen. Between the septa the convoluted tubes cut in all directions, some of them passing into narrow vasa recta, which converge to the corpus Highmori, where portions of the rete testis may be found. At the side of the testis, sections through the tubes of the epididymis will be seen, and probably the vas deferens.

With the high power it will be seen that the tubes of the testis appear almost full of round nuclei, except the central portion of some of the tubes, which is occupied by a colourless granular mass, consisting of the tails of the spermatozoa and coagulated fluid parts of the semen. The nuclei which lie next the wall of the tube are smaller and more deeply stained than those which lie more internally. Near the lumen of the tube are groups of short linear, deeply-stained bodies, arranged like the barbs of a feather. These are heads of spermatozoa still contained in their spermatoblasts.

The tubes of the epididymis will be seen to have a distinct lumen, partially occupied by a granular-looking mass (semen), in which are innumerable short, deeply-stained bodies (heads of spermatozoa). The epithelium is a single layer of columnar nucleated cells, each with an oval nucleus. The tube has a distinct muscular wall, in which the spindle-shaped nuclei will be recognised.

In the vas deferens the thick muscular coat will be noticed, and the mucous membrane probably thrown into folds, and consequently giving the cross section of the lumen a stellate shape.

Between the tubes of the testicle is the interstitial tissue, consisting, in the rabbit, mainly of lamellæ of connective tissue, with oval nuclei of endothelioid cells; but in the cat containing tracts of coarsely-granular cells, resembling those of epithelium.

2. To see the different layers of nuclei, the testicle of the rabbit is a very favourable object. A young adult should be chosen, and the testicle hardened in strong alcohol, as bichromate of potash destroys the nuclear figures. The sections should be extremely thin, stained in logwood, and mounted in balsam. Aniline dyes also give good results, but they are more difficult to use, and not so permanent as logwood. Next the wall will be seen one or two layers of deeply-stained nuclei, each presenting an irregular figure. Among these will be seen the large oval resting nuclei, feebly stained, and with distinct outlines (nuclear mem-

brane). More internally, two or more layers of nuclei, large, round, and presenting a beautiful network or convolution of coloured threads. Most internally, small, round, feebly-staining nuclei, with sharply-marked outlines. In some places these have become elongated: in others are groups of heads of spermatozoa still held in their spermatoblasts, which may in some places be traced to their attachment to the membrane of the tube. In other places free spermatozoa will be seen in the lumen of the tube. Rounded masses of protoplasm, each containing several nuclei of the third or inner layer, are commonly seen, apparently free, in the lumen of the tubes. These are the 'mother cells' of authors. They are probably the inner swollen ends of spermatoblasts detached by the section from the outer parts.

3. Testicle of rat. Müller's fluid; alcohol. This object is best cut by the collodion method of Matthias Duval, for by any of the ordinary means the section falls into pieces, since the tubes are held together only by a loose tissue, consisting of cells.

A small piece of the hardened testicle is transferred from alcohol to ether, where it is left for ten minutes. Then it is placed for some hours in collodion, and then in strong alcohol. The collodion is coagulated by the alcohol, but remains perfectly transparent. Sections can then be made, and stained in logwood, carmine, eosine, &c., but must be mounted in glycerine. The collodion does not require to be removed from the section, as it remains transparent and does not take the colour. It binds the tubes together, and gives the piece a suitable consistence for cutting. The method of embedding in celloidin, a later modification of the collodion method, will be described in the Appendix.

The seminal tubes of the rat are very large, and the spermatoblasts extremely conspicuous objects. They will be seen either as lobed bodies from which the tails of the spermatozoa have not yet grown, or in a more advanced form, when the hook-shaped heads of the spermatozoa lie in them, and the long tails project in a bundle. In this case the spermatoblast is short and contracted. Always between the spermatoblasts will be seen masses of coherent round cells. Where the spermatoblasts are immature, these round cells are small near the wall of the tube, but larger internally; but where the spermatozoa are about to be thrown off, the cells are much smaller and more numerous—that is, the nuclei have multiplied by division, so as to be ready to develop into spermatoblasts as soon as the spermatozoa of the former generation are thrown off. In the tubes of the rat's testis there are commonly seen rounded or irregularly-shaped masses, of a homogeneous appearance, and without nuclei. They are probably coagula.

4. Small bits of the testicle of the rat, hardened in Müller's fluid, are torn up in a drop of water or dilute glycerine. The isolated spermatoblasts will be seen, with their expanded foot,

containing a resting nucleus, and their divided inner end. In the more mature forms, the head, tail, and the protoplasmic swelling, corresponding to the lobe of the spermatoblast, will be seen, as well as isolated spermatozoa and round cells. It will be seen, as pointed out by Balbiani, that the round cells of the first and second layers are attached by processes to the walls of the tubes. This attachment is retained until they develop into mature spermatoblasts.

The cells of the interstitial tissue surround the blood-vessels. They are irregularly-shaped cells, with processes, by which the cells are united to one another in series.

5. A section is made into the epididymis of a recently-killed rabbit. Some of the milky fluid is pressed out, diluted with a drop of salt solution, and examined with a high power. The movements of the spermatozoa will be seen. Many of the spermatozoa present a little protoplasmic swelling at the junction of the middle piece with the tail. This corresponds to the similar swelling already seen in the spermatozoa of the rat.

Preparations of the spermatozoa of other animals should be examined—man, cat, frog, newt. The spiral filament will be seen in the newt.

6. Human **prostate,** hardened in alcohol. Sections should include the urethra. Logwood; balsam. Or double staining with eosine and logwood; balsam. The general substance of the organ will be seen to be composed of bundles of smooth muscular fibres, interlacing in various directions. These are stained red with eosine. Their nuclei, spindle-shaped when seen in length, round when seen in section, are coloured blue by logwood. Sections of the branching tubular glands appear as variously-shaped spaces, lined by columnar epithelium, which is blue. Some of the glands will be seen opening into the urethra. In some of the glandular spaces, concentrically-laminated concretions may be present. Sections of blood-vessels and nerves will be seen, and groups of ganglionic cells are commonly found.

7. Transverse section through the **penis** of a child. Vessels injected with Prussian blue; hardened in alcohol, or chromic acid and alcohol. Cochineal; balsam.

With the naked eye the section will be seen to consist of three parts—the two corpora cavernosa penis, and the corpus spongiosum, surrounding the urethra. With the microscope the strong tunica albuginea will be seen giving off the septa which divide the large blood spaces. In the septa and albuginea is much muscular tissue, recognisable by its spindle-shaped nuclei. In the interior of each corpus cavernosum will be seen the artery of the corpus cavernosum. The larger arteries run in the septa. They have very thick walls, and either directly, or more commonly after breaking into capillaries, they open into the large, irregular-shaped cavernous spaces, which are filled by injection, and whose walls are covered by an endothelium, the spindle-shaped nuclei of which can be seen.

The section of the urethra has a stellate or linear shape. The epithelium, except near the orifice, is columnar. Tubular glands may be seen opening into the urethra through the thick mucous membrane.

Pacinian corpuscles occur frequently along the course of the large nerves. Their structure will be described subsequently, with that of the skin.

CHAPTER XV.

FEMALE ORGANS OF GENERATION.

I.—*Ovary.*

THE surface of the ovary is covered, not by a flat endothelium, such as that which lines the general peritonæal cavity, but by a layer of columnar epithelial cells. The line around the attached margin of the ovary, where the endothelium and the epithelium meet, is sharp and well marked, and can be readily distinguished with the naked eye. On transverse section, the ovary is seen to consist of two portions—one, which passes from the attached edge or hilum into the interior of the organ, consists of loose connective tissue, and encloses the larger blood-vessels and lymphatics. It is named from its position the **medullary portion,** or, from its vascularity, the **zona vasculosa.** Surrounding the medullary portion, and reaching to the surface, is the **cortical portion,** or **zona parenchymatosa,** in which alone ova are contained. The **stroma** of this portion is formed of peculiar spindle-shaped cells, with long nuclei, and which lie in bundles. As to their real nature, great doubt exists, equally respectable authorities affirming, on the one hand, that they are connective tissue, and, on the other, that they are muscular. There occur also tracts of cells, resembling very closely the interstitial cells of the testicle, and which, like them, have a great proneness to contain fatty granules in their interior. These have been shown by Balfour to be of epithelial origin, and to be derived from the tubes of the Wolffian body. There is also a certain quantity of ordinary connective tissue, chiefly surrounding the blood-vessels.

Embedded in the stroma lie the **ova.** There is a portion of the stroma immediately under the epithelium which is free from ova. This is particularly distinct in the human ovary, where it consists of bundles of cells arranged in three layers—an outer and inner transverse, and a middle longitudinal. It is called the **tunica albuginea**; but it must be understood that it is not a membrane separable from the rest of the stroma, but merely the superficial part of this.

Underneath the tunica albuginea the ova are thickly placed, in some animals forming almost a continuous layer. These superficially-placed ova are mostly in an immature condition. Each ovum lies in a cavity in the stroma, which is not bounded by any distinct membrane. The ovum itself is a spherical cell, without evident cell-wall, consists of granular protoplasm, and contains a large vesicular nucleus, in the interior of which is a distinct nucleolus. The protoplasm of the ovum is called the **yolk,** the nucleus the **germinal vesicle,** and the nucleolus the **germinal spot.** The cavity in which the ovum lies is called the **follicle.** The ovum is surrounded by a single layer of flat cells, which separate it from the stroma. These cells appear spindle-shaped in section. They are the **epithelium of the follicle.**

Passing to the deeper parts of the ovary, we find the ova fewer, but more advanced in development. The ovum increases somewhat in size, and the epithelial cells which surround it increase in number, and become columnar in shape, forming a single layer of cells arranged radially about the ovum. A continued multiplication of the epithelial cells takes place, so that, as the development of the ovum advances, it comes to be surrounded by two, and finally by several, layers of cells. Those which lie next the ovum and those which line the interior of the follicle are columnar; the intervening cells are polygonal. Among these polygonal cells there appear clear round spaces, probably cells which have become vesicular, and the epithelial cells about these spaces arrange themselves radially, so as to form very beautiful figures. These clear spaces enlarge and coalesce, so that there is eventually found among the mass of epithelial cells of the follicle a considerable cavity, filled with albuminous fluid, in which some detritus of the cells still persists. This fluid is called the **liquor folliculi.** It lies between a layer consisting of several rows of cells, which lines the interior of the follicle, and which is called the **membrana granulosa,** and a mass of cells which surrounds the ovum, and which is called the **discus,** or **cumulus proligerus,** or **ovigerus.** This latter mass is not free, but is continuous at one side with the membrana granulosa, and at the other projects into the fluid which fills the interior of the follicle.

Before this stage of development is reached the follicle has got a proper wall, or **theca,** which consists of two layers—an outer, dense, and formed of concentrically-arranged fibrous tissue; and an inner, formed of looser tissue, in which are contained numerous cells, like white blood-corpuscles, and a plexus of capillary blood-vessels.

The ovum itself has increased in size, and, surrounding the yolk, there has been formed a thick clear cuticulum, or cell-wall, which on section appears as a bright border to the ovum, and is called the **zona pellucida.** This zona is perforated by minute radial pores, and has, consequently, a striated appearance. It is probably formed by the epithelial cells next to the ovum.

The mature follicle, consisting of its theca, membrana granulosa, liquor folliculi, and ovum lying in the cumulus proligerus, is called a **Graafian ve-**

sicle, or **follicle.** In the lower animals Graafian vesicles, with two ova, are very common.

The maturation of the ova takes place in the deeper parts of the ovary, and the follicle approaches the surface only when the ovum is about to be discharged.

When the discharge occurs the ovum, with the discus proligerus and the liquor folliculi, leaves the follicle, and is received by the open mouth of the Fallopian tube. The walls of the empty follicle contract, and the internal layer of the theca being less retractile than the outer layer, falls into folds. A process of growth then takes place in the inner cellular layer of the theca, which not only fills up the space rendered vacant by the discharge of the ovum, but gives rise to a large mass, which may grow to be more than half the size of the entire ovary. This is the **corpus luteum.** It consists of a sort of granulation tissue, composed of large cells, which contain a yellow pigment, and which have been classed by Waldeyer among that variety of connective-tissue corpuscles which he has named plasma cells. These cells are contained in the meshes of an abundant plexus of blood-vessels, surrounded by spindle-shaped cells, which form for them a sort of adventitia. What share the membrana granulosa takes in the formation of the corpus luteum is doubtful, but it is certainly subordinate to that taken by the wall of the follicle. The corpus luteum is a temporary structure, and assumes a much larger size, and persists a much longer time, when the discharged ovum has been impregnated, than when it has escaped this fate. Eventually the corpus luteum atrophies, and a fibrous scar is all that remains.

Many ova undergo a process of atrophy in the ovary, and are never discharged. The atrophy of the contents of the follicle is accompanied by a growth of connective tissue from its walls, by which the cavity is obliterated.

The larger **blood-vessels** lie in the medullary portion of the ovary. The arteries pursue a spiral course, and are known as *helicine arteries*. Small vessels only pass into the cortical portion, where they form a close network. The more immature ova have no special vessels, but in the internal layer of the theca of the larger follicles a very abundant capillary plexus exists.

The larger **lymphatics** lie in the medullary portion. There is said to be a network of lymphatics between the two layers of the theca of the larger follicles. Lymphatics are abundant in the corporea lutea.

The **nerves** are mostly non-medullated, and are probably mainly for the supply of the vessels. Fibres have been traced to the neighbourhood of the follicles, but their terminations are unknown.

In order to understand the nature of the ovum, and why the most deeply-placed ova are the most mature, it is necessary to give a very brief account of the development of the ovary. This organ consists at first of a connective-tissue ridge, which becomes the future medullary portion of the ovary, and is covered by a layer of columnar epithelium, the **germinal epithelium.** This epithelium proliferates, and forms several layers of cells. Some of these increase in size, and become spherical. These are the **primordial ova.** Outgrowths from the connective tissue extend into the epithelium, and, leaving always a growing layer of cells on the surface, include groups of the deeper cells. These groups are often elongated, and at a certain period of this inclusion they are still continuous with the epithelium of the surface. This has given rise to the idea that the ovary is a tubular gland, a view now no longer held. Eventually the groups of epithelial cells become separated from the surface, and divided up into smaller groups by complete walls of vascular connective tissue. Of the included groups of epithelium, the large spherical cells become the ova, while the other cells of the group become the epithelium of the follicle. Hence the ovum, like the spermatozoon, is an epithelial structure. Shortly after birth the inclusion of ova ceases. This seems to be due chiefly to the formation of the tunica albuginea, and it is probable that from this period no new ova are formed. The number of ova in the ovaries of a girl at birth has been variously calculated at from 400,000 to 36,000.

It is evident that the ova which lie deepest in the ovary are those which are first included. They are consequently the oldest, and this probably determines their more advanced development.

While there is no doubt as to the origin of the ova themselves, there is much difference of opinion as to the source from which the epithelium of the follicles is derived. Waldeyer and the majority of writers derive them from the surface epithelium, as described above. Kölliker traces them to the tracts of epithelial cells, which Balfour and others have shown to reach the ovary from the Wolffian body. Foulis believes that they are developed from the stroma of the ovary, while Harz believes that they are formed by proliferation from the ovum after its inclusion, to which origin Schäfer also in part attributes them.

II.—*Parovarium.*

The **parovarium,** or **organ of Rosenmüller,** in the human subject, lies in the broad ligament of the ovary. It is a fœtal structure, which in the female does not develop, but in the male sex becomes the epididymis. It consists of tubes formed of connective tissue, with a few muscular fibres, lined by columnar ciliated epithelium, and which contain a clear fluid.

In some of the lower animals the tubes of the parovarium lie in the connective tissue of the medullary portion of the ovary, and are continuous with the tracts of interstitial cells described above.

III.—*Fallopian Tube.*

The **Fallopian tube,** or oviduct, consists of a mucous, a muscular, and a serous coat. The tube is lined by ciliated columnar epithelium. The motion of the cilia is such as to produce a current downwards towards the uterus. The mucous membrane, composed of connective and elastic tissue, is thrown into longitudinal folds, which are much higher in the outer than in the inner end of the tube ; and in the former situation the folding is complicated by the existence of secondary folds on the larger primary folds. On cross section the depressions between the folds resemble tubular glands, but they are lined throughout by ciliated epithelium. The arrangement is analogous to that existing in the vas deferens. There is a muscularis mucosæ, composed of longitudinal fibres. The muscular coat consists of an inner thick circular layer and an outer longitudinal layer, which is thin and not continuous. The serous coat is connected to the muscular by connective tissue, mixed with elastic fibres. At the abdominal end of the tube the ciliated epithelium meets the endothelium of the peritonæum.

IV.—*Uterus.*

The **uterus** is lined by columnar ciliated epithelium, resting on a thick **mucous membrane**, composed of connective and elastic tissue, and containing numerous lymph corpuscles. The mucous membrane in the fundus and body has a smooth surface, but in the cervix it forms permanent folds—the plicæ palmatæ. Numerous long, branching, tubular glands, lined, like the general surface, by ciliated epithelium, open into the cavity of the uterus.

In the canal of the cervix the ciliated epithelium reaches down to a variable level, where it becomes continuous with the compound scaly epithelium which lines the vagina and the vaginal portion of the cervix. The level to which the ciliated epithelium reaches is lower in virgins than in those who have borne children. In the upper portion of the cervix there are minute acinose or short tubular glands, lined by ciliated epithelium, while glands are wanting in the lower portion, where the epithelium is scaly. The little vesicles which are known as the ovules of Naboth are probably glands whose secretion has accumulated in consequence of obstruction of the ducts. There is no proper submucous coat in the uterus or cervix.

The **muscular coat** is very thick, and consists of smooth muscular tissue exclusively. This lies in several layers, whose arrangement is very complicated. There is a tolerably well-defined external layer, in which the bundles run longitudinally; a middle layer, which forms the chief mass of the muscular coat, and in which the bundles are mixed with much connective tissue, and run in different directions, but mainly transversely and obliquely; and an internal layer, which is considered by many to belong to the mucous coat—to be, in fact, a very highly-developed muscularis mucosæ. Its bundles run longitudinally, and are situated just below the bases of the glands, between which they send processes. In the cervix there are three layers, the external and internal being longitudinal, and the middle circular. The muscular coat of the uterus is continuous with that of the Fallopian tubes, and extensions from it are continued into the round ligaments and the ligaments of the ovaries, as well as between the layers of the broad ligaments.

The **peritonæum** forms the external coat of the uterus.

The **blood-vessels** of the uterus are numerous. The most remarkable feature in their arrangement is the commencement of the veins in the middle muscular coat by dilated cavernous spaces, resembling those of erectile tissue.

The **lymphatics** form plexuses under the mucous and serous coats, and send numerous branches to the mucous membrane and muscular coat.

The **nerves** have numerous ganglia situated on their course. Other terminations than those in the muscular fibres are unknown.

V.—*External Organs.*

The **vagina** is a muscular tube, formed of an external longitudinal and an inner circular layer of smooth muscular fibres. The mucous membrane is lined by compound scaly epithelium, into which papillæ project. There are no glands, except at the lower part, in the vestibule, and about the opening of the urethra, where acinose mucous glands are found.

The **urethra** is lined in its upper half with transitional epithelium, which in its lower half becomes squamous. Numerous tubular glands open into the urethra. In the muscular coat there are dilated blood-vessels—a kind of corpus spongiosum.

In the **clitoris** and the **vestibule** is true erectile tissue, quite similar to that found in the male genital organs. In the clitoris the nerves terminate in genital corpuscles resembling those which have been described as occurring in the penis. On the labia minora are large subaceous glands, without hairs.

The **glands of Bartholinus** are analogous to Cowper's glands in the male, and resemble them in structure.

VI.—*Mammary Gland.*

The **mammary gland** is surrounded by a capsule of connective tissue, which sends septa through the gland, dividing it into lobes and lobules. There is also much fat on the surface of the organ and between its glandular parts. On the nipple fifteen to twenty large ducts open. Each of these, near its orifice, is dilated for some distance, so as to form a sort of sinus. These are much more developed in some of the lower animals than in the woman, and form receptacles for the milk. Each duct carries off the secretion of a lobe of the gland. The ducts have a wall formed of connective and elastic tissue, with smooth muscular fibres. The epidermis extends for a short distance into the ducts, but soon is replaced by a columnar epithelium, which is continued into the finer ducts, where it becomes reduced to cubical or flat cells. The ducts divide and subdivide, and the terminal branches open into the acini, which in the virgin are few, small, and lined by columnar epithelium, but in the pregnant or lactating woman are greatly increased in size and number. Under these circumstances each acinus is an elongated space, and appears, according to the direction in which the section is made, round or oval. Its wall is formed of a homogeneous basement membrane, strengthened by flat stellate cells placed on its inner side. The epithelium consists of a single layer. The cells may be flat, high columnar, or intermediate between these, the shape of the cells depending chiefly on the degree of distension of the acinus with milk, and the period which has elapsed since the last suckling (Heidenhain). The cells contain fat droplets, and similar droplets, each surrounded by an albuminous coat, lie free in the comparatively large lumen of the acinus.

It would appear that, in the formation of milk, the epithelial cells do not undergo destruction, but merely discharge the fat drops from their interior. The colostrum corpuscles are cells crowded with fat drops, and still containing their nuclei. They occur in the milk for a short time after delivery. Their occurrence was supposed to show that, in the formation of milk, the epithelial cells underwent a process of complete fatty destruction; but since cells similar to the colostrum corpuscles are never found in the epithelium of the acinus, it is probable that the colostrum corpuscles are amœboid cells, which have taken up into their interior the fat drops which had been discharged by the epithelium into the lumen of the acini.

The acini are surrounded by a plexus of capillary blood-vessels, and there is about each acinus a lymph sinus, which communicates with proper lymphatic vessels.

The terminations of the nerves are not known.

In the skin of the nipple and areola there is a considerable quantity of smooth muscular tissue.

1. Section of **ovary.** Best of cat or rabbit. Great care must be taken that the surface of the ovary is not touched or rubbed before hardening, as the epithelium is readily detached. The fresh organ should be suspended in Müller's fluid, and the hardening completed in the usual way by alcohol. Logwood; balsam.

Or the fresh ovary of a rabbit is placed for twenty-four hours in one per cent. osmic acid, well washed in water, and then hardened in alcohol. Sections mounted in glycerine. By this method the most beautiful results are got.

Observe the columnar epithelium of the surface; the superficial immature ova, each surrounded by a single layer of flat epithelial cells; the more advanced ova, surrounded by a single layer of columnar cells, and with commencing zona pellucida; the gradual increase of epithelial cells and formation of liquor folliculi up to the development of the perfect Graafian vesicle. Study each part of this as described above.

Observe the tissue of the ovary, consisting of spindle-shaped cells, with tracts of epithelioid cells frequently surrounding the follicles, and generally stained black in osmic acid preparations, in consequence of the contained fat.

The medullary portion of the ovary contains no ova, but large blood-vessels, the arteries often running a distinctly spiral course.

2. Section of the ovary from a fœtus, human or other mammal, hardened in Müller's fluid and alcohol. Logwood or picrocarmine; glycerine.

The germinal epithelium can be seen, according to the age of the fœtus, consisting of one or several layers of cells. Among the columnar cells others can be seen, which are larger, spherical, and with distinct nucleus. These are the primordial ova. Extensions of the epithelium can be seen dipping down into the tissue of the ovary, and consisting of groups of primordial ova, surrounded by masses of indifferent epithelium. In the deeper parts of the ovary the ova lie, each enclosed in its own follicle, and surrounded by epithelium.

Sections through the germinal ridge of the chick at the fourth or fifth day of incubation should be studied, in order to see the first formation of the ovary.

3. Section of the ovary of a pregnant cat, rabbit, &c. Müller's fluid; alcohol. Logwood; balsam or glycerine.

These ovaries contain corpora lutea, and the sections show the tissue of large granular cells set in a capillary network. In the recent corpora lutea of cows and other large animals, it is not uncommon to find crystals of hæmatoidin, arising from the blood which was extravasated when the follicle burst for the discharge of the ovum.

4. Cross section through **Fallopian tube.** Cat, bitch, or woman. Hardened in chromic acid and spirit. Logwood; balsam.

The lumen is stellate, in consequence of the prominent longitudinal folds of the mucous membrane, which are here cut across. The depressions between these look like glands. The epithelium is columnar ciliated. The muscular coat consists of two layers, the inner circular being much the thickest. Most externally the serous coat. Large blood-vessels in mucous coat.

5. In the human female the **uterus** is a single organ, but in most of the lower animals it consists of two tubes or horns, which coalesce only at their opening into the vagina, and each of which is continuous with the corresponding Fallopian tube. One of the uterine horns of a cat or rabbit is hardened in chromic acid and spirit. Sections are made transversely. Logwood; balsam.

The mucous membrane is thickly infiltrated with lymph corpuscles, and thrown into longitudinal folds, here cut across; the epithelium columnar; the cilia very difficult to see. From the surface open numerous tubular glands. These will never be seen in their entire length, since they run a very tortuous course.

Muscular coat.—The external longitudinal layer pretty distinctly marked, and all its fibres seen in cross section; the middle layer, consisting of fibres interlaced in different directions, mixed with connective tissue, and containing large venous sinuses; the internal layer sending bundles upwards into the mucous membrane.

6. **Mammary gland** of woman in later months of pregnancy. Harden in Müller's fluid. Sections washed in water, placed for a few minutes in one per cent. osmic acid, washed, stained in logwood or picrocarmine, and mounted in glycerine.

The acini will be seen as round or oval figures, each bounded by a basement membrane, and lined by epithelial cells, generally of cubical shape. The fat drops, stained black, are seen in the interior of the epithelial cells, and free in the lumen of the acini. Sections of ducts will be seen, smaller than the acini, and lined by low columnar or flat epithelium. Between the acini is only a comparatively small quantity of connective tissue.

If sections of the active mammary gland be mounted in balsam, the alcohol and oil of cloves dissolve out all the fat. The albuminous coat of the free fat globules can still be seen as faint circles, and the place of the fat in the cells is marked by spherical vacuoles.

Sections of an inactive gland should be compared with those from the secreting organ. The acini are smaller and fewer; the connective tissue relatively more abundant, and there is no fat in the epithelium.

CHAPTER XVI.

THE SKIN.

The skin, like the mucous membranes, consists of two parts, the true skin, cutis vera, dermis or corium, and the epidermis. The former is mainly composed of connective tissue; the latter is altogether composed of cells.

The **true skin,** or **corium,** passes without sharp line of demarcation into the subcutaneous areolar tissue. In this latter it has been shown that the connective-tissue bundles are loosely felted together, leaving between them considerable interspaces. In the skin this interlacement of the bundles is much closer, and becomes progressively more so up to the surface, the tissue in this way increasing in density. In the more superficial layers the bundles divide into their fibrils, which interlace with one another. In some places the deeper parts of the skin contain adipose tissue, disposed in little rounded masses; but the superficial parts of the skin are free from fat. The connective-tissue cells are flat, and lie between the fibrous bundles. Elastic fibres and networks exist abundantly in the skin.

From the surface of the skin numerous conical elevations, named **papillæ,** project into the epidermis. In most places the papillæ are small and irregularly scattered, but in others they are very large, and disposed with great regularity. This is particularly the case in the palms of the hands and soles of the feet, where the papillæ lie in rows, giving rise to the curved lines which are to be seen on these parts.

The surface of the corium is covered by a thin homogeneous layer, which is commonly described as a basement membrane, and is supposed to consist of flat endothelioid cells. The existence of such a membrane is, however, not universally admitted, and the homogeneous border is probably merely formed of the cementing substance which exists everywhere between the elements of the connective tissue (Unna).

The epidermis is composed of several superposed layers of cells, arranged generally in the manner of a compound scaly epithelium, but differing from the epithelia in that the cells of the superficial layer have become completely transformed into horny plates, and have lost their nuclei.

Four distinct layers can be recognised in the epidermis:

1. **The mucous layer, stratum mucosum, stratum Malpighii, stratum spinosum.**—This consists, first, of a single row of columnar-shaped cells, which rest on the basement membrane, or layer of cement, and are firmly fixed to this by teeth, which fit into depressions in its surface. Above this first row are several layers of polygonal cells, which all have nuclei, and a protoplasmic cell-substance, and are united to one another by numerous thin bands, which, when the cells are forcibly separated, appear as fine spines on their surface. From these spines the layer gets its name of stratum spinosum. It extends to a level somewhat above the tops of the papillæ. In negros and the coloured parts of the skins of whites the pigment is contained in the cells of this layer.

2. The **stratum granulosum.**—This consists of only two or three layers of cells, which are flattened parallel to the surface, and in vertical sections appear of a spindle shape. They are distinguished by containing coarse granules, which stain deeply in carmine and logwood. They are connected by spines, which are, however, much shorter than those

joining the cells in the stratum spinosum, so that the interspaces between the cells are narrower. The nuclei are small.

3. The **stratum lucidum** consists also of only a few layers of cells, which are flattened and horny, but which still retain vestiges of their nuclei. The cells are closely placed together, and on sections the outlines of the individual cells are often very indistinct, so that the entire layer has a homogeneous appearance. It stains a bright yellow in picrocarmine.

4. The **stratum corneum,** or **horny layer,** consists of thin, flat, horny scales, which have no nuclei, and have completely lost their cellular characters. They are more loosely attached to one another than are the cells of the deeper layers, and at the surface they are continually undergoing desquamation. According to Unna, the cells of this layer possess shrivelled nuclei, are still connected by spines, and the cornification is confined to the surface of the cells.

The thickness of the epidermis varies greatly on different parts of the surface of the body, and this is particularly true of the horny layer, which in some of the finer parts of the skin is only a very thin pellicle, but in others may attain a very great thickness.

The epidermis is much thicker between the papillæ than over their summits. This difference is due altogether to the stratum spinosum, which fills up the depressions between the papillæ. The other strata have in a given part of the skin almost a constant thickness.

The epithelium grows from below. The cells of the stratum spinosum multiply by division, evidence of which is found in the peculiar nuclear figures characteristic of dividing cells. The older cells are pushed upwards towards the surface, and when they reach the stratum granulosum there is formed in their interior a substance which occurs in the shape of coarse granules, which has been named eleidine by Ranvier, and which has been since carefully studied by Waldeyer, who calls it keratohyaline. This substance stains deeply in carmine and logwood, and it is to it that the opacity and consequent whiteness of the skin is due. Where it is wanting, as on the red border of the lips, on the nail-bed, on the mucous membranes, the surface is red, owing to the colour of the blood appearing through the transparent epidermis, or epithelium (Unna). It is believed by Ranvier and Waldeyer that the eleidine undergoes transformation, either of itself or after combination with the protoplasm of the cells, into keratin, which in the two upper layers of the epidermis almost completely replaces the original protoplasm of the cells. Unna, however, points out that while the eleidine is formed in the interior of the cells, the cornification affects their surface only, and in certain places cornification is not preceded by the formation of eleidine in the cells. Hence the connexion between this substance and keratin must be less close than is generally supposed.

The surface of the bodies of most animals is provided with peculiar appendages, which consist of modified epidermis. Such are, in the lower animals, scales, spines, feathers, hoofs, horns, &c. In man we meet with two such appendages, namely, the hairs and the nails.

The nails are flattened organs, free at their distal end, and inserted into a fold of skin at their proximal end. The fold is called the nail-fold or nail-groove. The lateral edges of the nail are overhung by folds of skin, called the nail-wall. The nail, except at its free end, rests on the nail-bed, which is formed by the dorsal surface of the phalanx, covered by the rete mucosum. The posterior part of the nail-bed is called the matrix. The portion of nail which corresponds to the matrix is called the root. The remainder is called the body. The root is thinner than the body, which is of the same thickness in its whole length. The nail consists of a number of layers of flattened horny cells, placed one over the other. These cells can be isolated by reagents, and are then seen to possess vestiges of nuclei.

The bed of the nail is formed of a dense corium, closely attached to the dorsal surface of the bony phalanx. The corium is covered by several layers of epidermic cells, corresponding in every particular with the stratum spinosum of other parts; and on this the horny nail rests directly, without the intervention of a stratum granulosum. Posteriorly the nail-bed passes into the matrix. This, which is the part from which the nail grows, is lenticular in shape. Its posterior boundary corresponds to the posterior edge of the nail, while anteriorly it is limited by a curved line, which separates the transparent distal part of the nail from the posterior opaque white portion, which is called the lunula. The matrix possesses a stratum spinosum; but interposed between this and the horny substance are several layers of cells, which have a granular appearance, and it is to these that the opacity, and consequent white colour, of the lunula are due. The nature of the granules is variously explained. Ranvier finds that they are due to a substance existing in the interior of the cells, which differs from eleidine in staining brown, not red, in picrocarmine, and which he calls, from its supposed function, onychogenous substance. Waldeyer, on the other hand, believes the granules to be formed of eleidine, while Unna thinks that the granular appearance is not due to anything in the interior of the cells, but to very thick spines, which cover their surface.

At the posterior part of the matrix the corium possesses long papillæ. More anteriorly these give place to ridges, which diverge from behind forwards, and which, when they reach the anterior edge of the lunula, turn forwards, and run parallel to one another as far as the distal end of the nail-bed, where they meet the ordinary papillæ of the skin of the finger. Corresponding to the ridges of the nail-bed are ridges on the under surface of the nail, which fit in between the former. The vascularity of the matrix and bed of the nail is very great, numerous capillary loops passing into the papillæ and ridges of the corium. Besides the usual communication by means of capillaries between the veins and arteries, these vessels communicate directly with one another (Hoyer).

The nail grows exclusively from the matrix, and is pushed forwards over the cells of the bed, but receives no increase in thickness from this part (Unna). The nail is originally formed underneath the horny layer of the epidermis, and part of this horny covering, or eponychium, persists throughout life, and projects over the posterior part of the nail for a certain distance.

The hairs are cylindrical or very elongated conical bodies. Each hair consists of a free portion or shaft, and of a root, which is implanted into a depression in the skin.

A hair has a cellular structure. It is covered externally by thin, flat, non-nucleated, horny scales, which are of an oblong shape, with their greatest length placed transversely to the axis of the hair, and which are imbricated, each scale slightly overlapping that which lies above it. These scales constitute the **cuticle of the hair.** Inside the cuticle are more or less spindle-shaped cells, which possess rod-like nuclei, and which constitute the **shaft** of the hair. In coloured hairs these are pigmented, and there is also pigment contained in the cement substance which holds them together. In the axis of the hair there are found roundish or polygonal cells, often separated by spaces containing air. These constitute the third element, **the pith** or **medulla,** of the hair. They are not present in all hairs.

Before describing the root of the hair, it will be necessary to give some account of the depression into which it is implanted. This is called the **hair-follicle,** and is a tubular depression of the corium and of the stratum spinosum of the epidermis. It is generally placed obliquely to the surface. The follicles vary extremely in depth. Those of fine hairs are situated altogether in the true skin, while those from which grow the coarse hairs of the head, beard, &c., extend far down into the subcutaneous areolar tissue. Each hair-follicle opens on the surface by an expanded orifice, called the **mouth.** Deeper, a little below the level of the general surface of the true skin, it is narrowed. This narrowing is the **neck.** Beyond this it again becomes wider, and forms the **fundus.** Into the bottom of the follicle projects a conical **papilla,** with a somewhat constricted base, and which is in every way analogous to the ordinary papillæ of the surface.

The follicle is bounded externally by a layer of connective tissue, whose bundles run parallel to the long axis of the follicle. Internal to this is a thicker layer of connective tissue arranged transversely, and with which is mixed a certain quantity of smooth muscular fibres. Internal to this is a homogeneous vitreous layer or basement membrane, most distinct at the deeper part of the follicle.

All the layers of epidermis extend into the mouth of the hair-follicle, but from the neck downwards the arrangement becomes different. In the middle part of the follicle we find the stratum spinosum, which has been continued from the general epidermis, but which here receives the name of the **external root sheath.** It resembles in many respects the stratum spinosum of the surface. It rests on the basement membrane by a layer of short columnar cells; more internally the cells become polygonal, and are united by processes or spines; while the most internal layers are somewhat flattened. But it differs fundamentally from the general stratum spinosum in this—its cells never develop into a stratum granulosum or become horny. In the lower part of the follicle the external root sheath becomes greatly diminished in thickness, and, reduced to its deepest layer of columnar cells, it terminates close to the base of the papilla.

Internal to the external root sheath, between it and the hair, we find the **internal root sheath.** This consists of only three layers of epidermic cells. The most external is called **Henle's layer,** and is formed of oblong flat cells, usually without nuclei. The second layer is called **Huxley's layer.** Its cells are somewhat thicker than those of Henle's layer, and are commonly nucleated. Most internally is a layer of very thin, flat, non-nucleated scales, forming the **cuticle of the hair-follicle,** and which are in apposition with the outer surface of the hair. The internal root sheath terminates above at the neck of the follicle, and is not continuous with any of the epidermic layers which cover the surface of the skin. The following Table gives all the structures met with in passing from the periphery to the axis of a hair-follicle :—

1. Longitudinal layer of fibrous tissue,		Connective tissue,	Follicle.
2. Transverse layer of fibrous tissue,			
3. Vitreous layer, or basement membrane,			
4. External root sheath,		Epidermis,	
5. Internal root sheath,	Henle's layer,		
	Huxley's layer,		
	Cuticle of hair-follicle,		
6. Cuticle of hair,		Hair.	
7. Cells of shaft of hair,			
8. Medulla, or pith of hair,			

In the upper part of the follicle the hair has the same structure as in its free portion, but at the deeper part it expands into a mass of polygonal-shaped cells, which fill the lower part of the follicle, and into the interior of which the papilla projects. This is called the **hair-bulb.** The cells which rest immediately on the papillæ are, with the exception of those at its apex, all columnar in shape. From the cells about the base of the papilla are developed, lowest down, the internal root sheath; from those higher up, the cuticles of the follicle and of the hair. From the cells at the side of the body of the papilla is developed the shaft of the hair, while from those at the apex the medulla of the hair arises. The cells of the internal root sheath contain eleidine in large quantity. This disappears as the cells rise up in the follicle, but is retained to a much higher point by the cells of Huxley's layer than by those of Henle's. The medulla of the hair also contains at first much eleidine, while this substance is altogether wanting in the cells of the cuticles and in those of the shaft of the hair. Between these latter cells there is a large quantity of pigment, varying in amount and colour according to the colour of the hair. At a short distancefrom the papilla the cells of the different layers begin to assume the shape proper to each.

The hair is thus an epidermic structure, developed external to the basement membrane; and the internal root sheath has no analogue in the epidermis of the general surface, but, like the hair itself, grows from the mass of cells which form the bulb and lie on the outside of the hair-papilla.

So long as the hair continues to grow, it maintains its connexion with the papilla, and is in this condition called a **papillary hair,** or a **hair with a hollow bulb.** The duration of the life of a hair is, however, limited, and sooner or later the cells over the papilla cease to grow, and those already formed become horny. The hair is thus separated from the papilla, and is gradually pushed upwards in the follicle, the lower portion of which, including the papilla, undergoes atrophy. For a time the hair remains with its deep end entangled in the cells of the external root sheath of the upper part of the follicle. It now has ceased to grow, it has no internal root sheath, and its end is solid and club-shaped, or split into fibres, which dovetail with the cells of the external root sheath. It is now called a **hair with a solid bulb,** or a **bed-hair,** or an **intercalated hair.** It soon falls out; but, before it does so, a new hair, developed either from the old papilla, or from the papilla of a new follicle, itself an outgrowth from the old one, makes its way into the latter, and we find projecting from the same orifice on the surface two hairs, one seated on a papilla, the other embedded among the cells of the external root sheath. Some observers believe that the bed-hair, after it has taken root among the cells of the external root sheath, still continues to grow for a time.

From the neck of the hair-follicle there comes off the **sebaceous gland.** This consists of a short duct, which divides into a certain number of dilated pouches or acini. The wall of these is formed by a continuation of the vitreous layer of the hair-follicle, and the epithelium is continuous with that of the external root sheath. The acini are filled with cells. Those near the basement membrane are nucleated and protoplasmic, while those in the central parts of the acini have broken down into a mass of fatty granules. All stages in the fatty degeneration of the cells can be seen. The secretion of the glands consists mainly of the fatty detritus of the epithelial cells, which do not form the fat in the way in which it is formed in the mammary gland, but themselves break down into the material of the secretion.

Usually the sebaceous glands open into the neck of the hair-follicles; but in some places, where the hairs are small or absent and the sebaceous glands large, these latter may open on the surface. It has been already mentioned that sebaceous glands occur in certain parts where there are no hairs. This is the case at the different mucous orifices of the body, as the red border of the lips, the labia minora, and the inner surface of the labia majora in women, the prepuce and glans penis (Tysonian glands), and the edges of the eye-lids, where the Meibomian follicles must be considered as modified sebaceous glands.

Attached to the exterior of the hair-follicle, on that side at which it forms with the deep surface of the skin an obtuse angle, are one or more bundles of smooth muscular fibres, which pass towards the surface, and are inserted into the connective tissue of the true skin. In this course they pass below the acini of the sebaceous glands, and serve to press out their secretion, as well as to pull on the hair-follicle, and so diminish the obliquity of the hair. From this latter function these little muscles are called the **arrectores pili.**

Besides these muscles, there exists in many parts of the skin a considerable quantity of smooth muscular tissue, forming networks parallel to the surface. This tissue has been already mentioned as occurring in the skin of the nipple and areola of the breast. It is found also in the scrotum and penis and other parts.

There is a very close connexion between the muscular and elastic tissues in the skin, by means of which the contraction of the muscular fibres is more widely and equally effective than if such a connexion did not exist (Unna).

The **sudoriparous** or **sweat glands** exist in most parts of the skin, and are especially numerous in the palms of the hands, soles of the feet, and axillæ. Each gland is composed of a single tube, which opens on the surface by one extremity, runs a pretty straight course through the skin, and in the subcutaneous tissue, or deeper parts of the true skin, is twisted into a coil, and terminates by a closed end.

In the straight portion the tube is composed of a basement membrane and an epithelium, consisting of two or three layers of cubical, or somewhat flattened cells. The cells which bound the lumen of the tube have a shining border, or cuticulum, on their free edge. The tube retains this structure for some distance after it has begun to twist into the coil, but then undergoes a marked change. It becomes much wider, and, while still retaining its basement membrane, its epithelium becomes reduced to a single layer of cells. These are pyramidal, clear, with round nuclei, and present at their outer ends a radial striation, something like what is observed in the cells of the renal tubules. Between the basement membrane and the epithelium is a layer of smooth muscular fibres, placed obliquely to the long axis of the tube. These muscular fibres are developed from the upper germinal layer of the embryo, and they are the only example of muscle having this origin, and lying superficial to the basement membrane (Ranvier). The dilated portion of the tube of the sweat gland is considered to be the secreting part; the narrower portion is the duct. This latter reaches the surface of the corium always between two papillæ. Here it loses its basement membrane and proper epithelium, and, pursuing through the epidermis a spiral course, is bounded only by the cells of the different strata through which it passes. These strata all dip down slightly around the tube as it passes through the epidermis.

The **ceruminous glands** of the external auditory meatus resemble the sudoriparous glands.

The **blood-vessels** of the skin are not uniformly distributed, but each of the many structures which exist in this part has its own special vascular system.

There is an abundant distribution of blood-vessels to the superficial part of the true skin, and capillary loops pass into the papillæ. The coils of the sweat glands are surrounded by a close capillary network, and along the ducts vessels run which establish a communication between the superficial plexus and that of the gland. The hair-follicle has also a special network of capillaries, and a small artery enters and forms a loop of capillaries in the papilla of the hair. Connected with the vessels of the hair-follicle are networks surrounding the sebaceous glands, and supplying the arrectores pili muscles.

The fat in the skin is, like adipose tissue in other parts, provided with a close capillary plexus surrounding the cells.

The direct communication between the arteries and veins at the extremities of the fingers has been already mentioned. A similar communication occurs in animals in the ears, tail, snout, and, in fact, in all projecting parts of the body (Hoyer).

The **lymphatics** of the skin are very numerous. They form horizontal plexuses, from the superficial of which offsets pass into the papillæ. The lymphatics originate between the connective-tissue bundles as splits or fissures, which are the juice canals of the part. These communicate, on the one hand, with the true lymphatic vessels, which have proper walls and valves; and, on the other, with the interspaces between the epidermic cells of the stratum spinosum and of the external root sheath, which interspaces must thus be looked on as part of the lymph-canalicular system of the skin (Key and Retzius. Klein. Unna). The sweat glands are surrounded by lymphatic sinuses or splits, and similar fissures are also found between the bundles of the arrectores pili muscles, and in the adipose tissue surrounding each fat cell (Klein).

The **nerves** of the skin are very numerous, and terminate in different ways.

A certain number of fibres, having lost their medullary sheath, penetrate the basement membrane, and ramify as naked axis cylinders in the stratum spinosum of the epidermis, where they terminate by free ends below the level of the stratum granulosum. The nerves are generally believed to lie altogether between the epidermic cells; but it has been recently affirmed that the terminal fibres penetrate the cells, and end by minute swellings just exterior to the nucleus (Pfitzner. Unna). This statement is much in need of confirmation. Peculiar branching bodies are present among the epidermic cells, and were, when they were first discovered, supposed to be connected with the nerve fibres as terminal organs. This is now known not to be the case; but the real nature of the bodies in question is still unsettled. Ranvier holds them to be wandering cells. Klein looks on them as connective-tissue corpuscles, which here, as in other epithelial structures, lie among the epidermic cells.

Hairs are abundantly supplied with nerves, which are all distributed about the neck of the follicle. Here a number of naked axis cylinders come off from medullated nerve fibres, and penetrating the vitreous membrane, terminate internal to this, apparently by free ends. There are no nerves in the papilla, or the lower parts of the follicle.

Many of the lower animals have hairs, which serve as tactile organs, such as the whiskers of cats, rats, &c. These tactile hairs are surrounded by a sheath of erectile tissue, which is contained between the layers of the follicle, and they have a great number of nerves distributed to them. The fibres terminate within the vitreous membrane and at the upper part of the follicle. The exact mode of their termination is not certainly known; but it would appear, that while some end in menisci, which are in connexion with tactile cells, others terminate by free ends (Ranvier).

Besides the termination by free ends, the nerves of the skin terminate in peculiar structures, which are termed end organs. Of these, the tact corpuscles of Meissner and the Pacinian bodies are best known.

The **tact corpuscles of Meissner** are oval bodies, which lie in the papillæ of the skin, and are particularly well developed and numerous in those parts where the tactile sensibility is most acute, as the palmar surfaces of the fingers and toes, the lips, &c. Each corpuscle is so placed that its long axis is parallel to that of the papilla in which it lies, and which it nearly fills. The corpuscle sometimes is single, sometimes consists of two or more lobes placed one over the other. It is enclosed in a connective-tissue capsule, and possesses some nuclei of its own, which all lie near the periphery. To each lobe at least one medullated nerve fibre goes, and, according to Ranvier's most recent observations the nerve ends by giving off twigs, which break up into little bunches of terminal fibres, which are placed at right angles to the long axis of the corpuscle. The development of the tact corpuscles shows that they are composed of a number of peculiar cells of connective-tissue origin, between which the terminal nerve fibres are placed.

In some parts of the epidermis there are found large, clear, oval cells, which were supposed at first to form the terminal organs of nerve fibres (Merkel). Ranvier has, however, shown that the nerve fibres do not terminate in these cells, but in a thin concavo-convex body, or **meniscus,** in the concavity of which the large oval cell is placed. This latter is analogous to one of the cells of a corpuscle of Meissner. In other organs, in the lower animals, a similar connexion is found between a true nerve termination and peculiar cells which are connected with it in the same end organ, but with which the nerve fibre has no direct continuity.

The tactile menisci are more numerous and better developed in the lower animals than in man. They are best seen in the external root-sheath of the tact hairs, and in the epidermis of the snout of the pig.

Pacinian corpuscles occur in the subcutaneous tissue of many parts, particularly along the nerve trunks in the fingers and toes. They are found also about joints, along interosseous membranes, and connected with the ramifications of the abdominal sympathetic nerve. In the cat they exist in enormous numbers in the mesentery of the small and large intestine and in the pancreas.

A Pacinian corpuscle is an oval body, and consists mainly of a number of capsules, or lamellæ, lying one inside the other. Each lamella is formed of connective tissue and fine elastic fibres, which run, some parallel to the long axis of, others at right angles to the corpuscle. The fibres are separated by serous fluid. Each lamella is clothed on both surfaces by endothelial cells, whose nuclei are easy to see, and it is the lines of endothelial cells which serve best to mark out the limits of the lamellæ. Since neighbouring lamellæ lie in contact, the two layers of endothelium which are in apposition form apparently a single line. The clear intervals between these lines are the lamellæ themselves, whose thickness is mainly due to the serosity which is contained between the fibres of which they are composed. The outer and inner capsules are thinner than those between. It will be seen that the structure of the capsules resembles closely that of the lamellæ of which the perineurium of the nerves is composed; and, in fact, the capsules are derived mainly from the lamellæ of the perineurium of a nerve fibre, which enters each corpuscle at one of its small ends. The lamellæ of the perineurium become thickened, and pass away from the fibre one by one, to form the capsules of the corpuscle. So long as the capsules are being thus formed, the nerve fibre retains its white substance, but loses it when the last lamella is given off, and then runs as a non-medullated fibre in the axis of the corpuscle, enclosed in a mass of substance whose real nature is still uncertain, and which is clothed by the capsules. Arrived at the distal end of this core, the nerve fibres terminate by a swollen end, or break up into a number of branches, each of which so terminates. Sometimes a nerve fibre passes right through one Pacinian corpuscle, to terminate further on in another. In such a case the fibre loses its white substance, while it is passing through the core of the first corpuscle, but regains it again on leaving the latter.

The Pacinian corpuscles are supplied by blood-vessels, which ramify in the capsules.

There are many other organs in which the sensitive nerves terminate in the skin and other parts, but there is still much uncertainty as to their true structure. The genital corpuscles have been already alluded to, and Krause's corpuscle will be described with the conjunctiva. Transitional forms between Pacini's and Krause's corpuscles have been recently described by Golgi as occurring at the junctions of muscles with their tendons.

Portions of skin may be hardened by any of the usual methods: alcohol; chromic acid and alcohol for ten days, then alcohol alone; Müller's fluid a month, and then alcohol. But the best method of preparing the skin of the finger is to include in a ligature the whole root of the finger, with the exception of one of the collateral arteries, and then to force into this a one per cent. solution of osmic acid. The artery being tied, the finger is left for an hour, when it will have assumed a dark colour. Portions of the skin and subcutaneous tissue may then be removed, and placed in alcohol (Ranvier). The skin is a difficult object to cut by hand, as its numerous constituents differ very much in consistence. This difficulty may be overcome by soaking the piece to be cut for some hours in strong gum, and then placing it for twenty-four hours in alcohol. The alcohol hardens the gum, and gives the entire piece an even consistence. Before staining, the sections must be placed for some time in water, to remove the gum. Good sections can also be readily cut by the freezing microtome.

1. Vertical sections through the skin of the palmar surface of the finger prepared in one of the above ways; stained in logwood or picrocarmine; mounted in glycerine or balsam.

The different layers of the epidermis will be seen. The stratum corneum usually presents a very remarkable appearance, being in picrocarmine preparations variegated red and orange, and in logwood blue and yellow. The stratum lucidum in picrocarmine is bright yellow; the stratum granulosum red. In logwood these layers are unstained and blue respectively. In the stratum spinosum the polygonal shape of the cells and the fine serration around their edges will be seen in good sections.

The papillæ are large and regularly placed. In each ridge on the skin there are two rows of papillæ; so that if the section be across the ridge the papillæ will be seen in pairs, and between the pairs all the layers of the epidermis dip slightly downwards. But it is the stratum spinosum which fills up the valleys between the papillæ. In some of the papillæ blood-vessels will be seen, particularly in the osmic acid preparations, where they remain distended; in others, Meissner's tact corpuscles. On these are transverse markings, due to the windings of the nerve fibres and to the transversely-placed nuclei. In osmic acid preparations the black-stained nerve fibres can be traced to the corpuscles.

Many small nerve trunks will be met with, and their thick perineurium will be well seen in cross sections.

In the deeper parts, sections of the lamellated Pacinian corpuscles may be found. They are readily recognised by their concentric lamellæ, on which can be seen the oval nuclei of the endothelium. The coils of the sweat glands are very numerous. In each coil the tube will be several times divided by the section, and will appear, sometimes in length, sometimes in transverse section. The two portions of the tube forming the coil will be seen, and the details of the structure of each should be noted. The duct, as it runs a somewhat tortuous course, will generally not be seen in its whole length. It reaches the surface of the true skin at the bottom of the valley between two papillæ, and winds in a spiral course through the epidermis. This spiral passage is best seen in the skin of the heel, where the horny layer of the epidermis is very thick.

The fat, bundles of connective tissue, and numerous arteries and veins, do not differ from those met with in other parts.

2. Section through the skin of the finger, whose vessels have been injected with Prussian blue. Picrocarmine or cochineal; balsam.

The network of vessels under the skin, the loops in the papillæ, the plexus about the sweat glands, and in the fatty tissue will be seen. Elsewhere the vascularity is not very great. Each duct of a sweat gland is accompanied by two or more vessels, which communicate between the superficial plexus and that about the gland. In sections of a Pacinian corpuscle it will be seen that the vessels run in the outer capsules.

3. Vertical section through the skin of the scalp, made parallel to the direction of the hairs. Logwood or picrocarmine; balsam.

The epidermis is very thin as compared with that of the finger, particularly the horny layer. The papillæ are stunted and broad. The hairs are set obliquely to the surface, and most of them will be cut obliquely, and only fragments of them will be found, but some of them will be cut in their entire length. The follicle will be seen to open on the surface by a trumpet-shaped mouth, into which all the layers of the epidermis extend. From the narrowed neck the sebaceous gland will be seen to come off. Below this the hair is bordered by a shining, colourless line, which is the internal root sheath, whose different layers are most distinct in the deeper parts of the follicle. Between this and the wall of the follicle will be seen the much broader external root sheath, which resembles the stratum spinosum of the epidermis.

At the bottom of the follicle the hair will be seen to expand into the bulb, a mass of cells into which the conical papilla projects. In some follicles, hairs with solid bulbs may be seen implanted in the external root sheath. Below the hair the atrophied follicle extends. Surrounding the lower parts of the hair follicles is a quantity of adipose tissue, and extending obliquely from the side of the follicle to the surface will be seen the arrector pili muscle.

Sweat glands are numerous in the scalp. They resemble in structure those in the hand, but are smaller and more superficially placed.

4. Transverse sections through hairs and their follicles. Logwood, picrocarmine, or cochineal; balsam.

These sections must not be made parallel to the surface of the skin, since the hairs are implanted obliquely. The direction of the hairs must be determined, and the section made accurately at right angles to this. The different layers of the wall of the follicles and the root-sheaths can be well seen in these sections. Huxley's and Henle's layers can be readily distinguished. The appearances will differ according to the depth at which the hair follicle is cut. They will be readily understood by referring to the description of these parts.

It will be noticed that the hairs are not uniformly distributed, but that they occur in groups, with wider spaces between. The other tissues of the skin will be readily recognised.

Very beautiful preparations of the skin may be made by first staining the section in picrocarmine, then in a watery solution of aniline violet until the section is almost black. The section is then placed in a watch-glass with strong alcohol, and moved about with a needle until almost all the blue colour is removed; then passed rapidly through oil of cloves, and mounted in balsam. The process is a little difficult, but, when successful, gives admirable results. The connective tissue is red, the internal root sheath bright blue, the other epithelial structures orange, and the horny tissues yellow. Most of the nuclei are violet.

5. A hair examined in a drop of water shows, on careful focussing, fine lines running across it, which are the outlines of the cells of the cuticle. At the edge a fine serration may be seen, due to the imbrication of these cells.

If the hair be placed on a slide, in a drop of strong sulphuric acid, and slightly warmed, by tapping on the cover-glass the cells can be isolated. The non-nucleated flat cuticle cells and the spindle-shaped cells of the shaft still retaining vestiges of nuclei will be seen.

If a hair be pulled out, it will almost always bring the internal root sheath with it. The cells of this may be isolated by sulphuric acid or a strong solution of potash, and their shape and arrangement studied. In torn-out hairs the proximal ends differ in appearance, according as the hair was a papillary or a bed-hair. In the former case the bell-shaped hollow which received the papilla can be seen, while in the bed-hairs the end is club-shaped, and generally surrounded by a mass of the cells of the external root sheath, which is torn out with it.

6. In long and transverse sections through the nail and its bed, the appearances will be readily understood from the description of the nail already given.

The nail-cells may be isolated by placing fragments of a nail in thirty-three per cent. caustic potash for an hour, keeping the vessel in which the solution is contained carefully covered. It is easy then to isolate with needles the cells, which are now swollen, and show in their interior atrophied nuclei.

7. Pacinian corpuscles are best got from the mesentery of the cat. They appear among the fat as oval transparent bodies, readily visible to the naked eye. In the meso-rectum there is not so much fat about them as in the mesentery. They may be dissected out and examined without the addition of any reagent, or in salt solution. In this way their structure is most beautifully seen.

They may be hardened in one per cent. osmic acid and alcohol, and imbedded, when long and transverse sections can be made, and mounted in glycerine.

A fresh Pacinian corpuscle placed for a few minutes in nitrate of silver, one-half per cent., washed and mounted in glycerine, shows, after exposure to the light, the outlines of the endothelial cells which line the capsules, and which are now marked out by fine dark lines.

The nerve terminations in the epidermis can be made out only by staining in chloride of gold. The process is too difficult to be attempted by beginners.

CHAPTER XVII.

THE EYE.

I. **The Eyelids**.—The **skin** of the eyelids is delicate, and the subcutaneous tissue loose and almost free from fat. Beneath the subcutaneous tissue is the **orbicularis palpebræ muscle,** whose transversely-striated fibres run parallel to the surface. Next to this is found the so-called **tarsal cartilage,** which is not cartilage of any form, but a firm connective-tissue plate. Closely attached to this is the **conjunctiva,** a firm connective-tissue membrane, covered by an epithelium consisting of three or more layers of cells, the superficial of which are short columnar bodies.

At the free edge of the lids we find the large hairs forming the **cilia** or eyelashes. These are cast off and renewed with great frequency, so that the old cilia are very often seen in the stage of bed-hairs, while all the steps in the development of young hairs can be well studied in the eyelids. Each hair is, like similar structures in other parts, provided with sebaceous glands. Behind the cilia open the **glands of Moll:** these are similar in structure to sweat glands, but the glandular tube does not form a coil, and their ducts frequently open into those of the sebaceous glands of the cilia. Behind Moll's glands and near the posterior part of the edge of the lids are the openings of the **Meibomian glands.** Each of these glands consists of a long straight tube, from the sides of which open off a number of rounded or branched acini. They lie embedded in the tarsal plate, and resemble in most respects the sebaceous glands. Their epithelium is a continuation of the stratum Malpighian or spinosum, and the inner cells of the acini break down by fatty degeneration, as do those of the sebaceous glands.

At the edge of the lid, lying mostly in front of, but partly behind, the openings of the Meibomian glands is the innermost portion of the orbicularis palpebræ, or the **musculus ciliaris Riolani.**

II. **The conjunctiva** is a thin connective-tissue membrane covered by a compound epithelium, whose superficial cells are of low columnar shape or approaching flat scales. On the tarsal conjunctiva there are some papillæ which increase in size towards the line of reflexion, but are absent on the bulb. The sub-conjunctival tissue is loose except on the tarsal cartilage. Where the conjunctiva is reflected from the eyelids to the globe of the eye there are small mucous glands, most numerous in the upper lid. There is also more or less adenoid tissue in the conjunctiva, which sometimes is collected into follicles. The blood-vessels are numerous, particularly the veins.

The **caruncula lachrymalis** contains acinose glands, and some hairs grow from it.

The nerves of the ocular conjunctiva terminate in the **end bulbs of Krause.** In the lower animals, as the calf, each of these is a long oval body, surrounded by a laminated sheath somewhat resembling that of a Pacinian corpuscle, but consisting of only a few layers. Inside this is the core, which is finely granular or longitudinally striated. The nerve fibre enters the core at one end, and, losing its medullary sheath, runs to the opposite end, where it terminates in a slight swelling. In men the end bulbs appear to have a more complicated structure. Inside the laminated sheath is what at first sight seems to be a number of cells, and which are very generally supposed to be such. The nerve fibre loses its medullary sheath, and penetrates the end bulb, but divides repeatedly, and runs a tortuous course within this body. According to some authors, the terminal branches of the nerves end in the supposed cells: according to others, between these; while Schwalbe is of opinion that the nerve fibre becomes surrounded by a sheath, like the core of the simple end bulbs, which core, like the fibres themselves, branches and pursues a tortuous course, and that the supposed cells are merely this sheath seen in cross section, and that what have been taken for the nuclei of the cells are the cross sections of the nerve fibre in the centre of its sheath.

III. The **lachrymal gland** resembles in structure a serous salivary gland.

IV. **The Cornea.**—The conjunctiva ceases at the margin of the cornea, its tissues becoming continuous with those of the latter.

The epithelium of the cornea is compound scaly. The deepest cells are very long, and have each an expanded foot by which it rests on the anterior elastic lamina, and a rounded superficial end. Their sides are marked by ridges and depressions caused by the pressure of neighbouring cells. The cells of the next layer have an irregular shape, and are deeply excavated on their lower surface, so as to rest on the club-shaped ends of the deeper cells. Above this the cells grow progressively flatter, and in the superficial layers are thin scales. In the middle layers the cells are connected to one another by minute bridges, as in the case in the stratum spinosum of the epidermis. **The proper tissue** of the cornea is formed of a number of superposed lamellæ, which can be easily separated from one another. The **lamellæ** are composed of extremely fine fibres, held together in bundles by a cementing substance similar to that which unites the fibres of ordinary connective tissue. A similar cement unites the bundles, and even the lamellæ. The fibres in the same lamellæ run for the most part parallel to one another; but those of adjacent lamellæ cross each other at right angles, so that on a section through the cornea we meet with layers of fibres cut across alternating with those in which the fibres are cut in length, very much as was the case in the lamellæ of compact bone. There are some bundles of fibres which pass from one lamella to another. These connecting bands are better developed in the cornea of man and mammals than in those of lower animals, so that the lamellar structure is less distinct in the former than in the latter (Schwalbe). Between the lamellæ are the **cornea corpuscles** or **cells.** These are flat, branched bodies, which anastomose by their processes, and thus form a cellular network. Each cell consists of a finely granular protoplasm surrounding a very flat but large nucleus, which is often lobed or of irregular figure, but whose general shape is oval. The cells present, on their surfaces, ridges separating depressions which are produced by the pressure of the adjacent fibrous bundles, as in the case in the cells of tendons and fasciæ.

The cornea corpuscles lie in cavities in the cement substance which unites the lamellæ. These cavities are generally considered to be somewhat larger than the cells they contain, and to furnish the channels through which the lymph of the cornea circulates. They are, in fact, the juice canals or lymph-canalicular system of the cornea. A different view as to the arrangement of the cornea cells is held by Schwalbe. According to him the branching and anastomosing figures which can be got by treating the cornea with silver or gold salts, are not, as is commonly supposed, cells, but spaces communicating with one another by canals, and forming a system quite analogous to the lacunæ and canaliculi of bone. This system of canals is bounded by a membrane, formed of a horny material, and which is firmer and more resistent than the tissue which forms the corneal lamellæ. The canals are filled by plasma or lymph, and constitute the lymph-canalicular system of the cornea. In the widened nodal points of the canals are situated the cells. Each of these is a thin, flat plate which is protoplasmic about the nucleus, but at the edges thins out to a delicate horny film. The cell lies, like an endothelium, close against one wall of the flattened space which contains it, and the margin of the cell becomes continuous with the horny wall of this space. In some animals each widened portion of the canalicular system contains but one cell: in others several cells may lie in each space. In this case the cells meet each other edge to edge like an endothelium—in fact, the widened portions of the lymph canalicular system, which are flat branched spaces, have on one side an endothelial lining, while on the other side this is wanting.

Besides the branching connective-tissue cells, which are fixed in their position, the cornea contains a variable number of **wandering cells,** whose shape alters as they squeeze themselves through the narrow passages between the bundles of fibres and lamellæ.

The anterior portion of the cornea subjacent to the epithelium differs somewhat from the remainder, and is generally described as a distinct layer, under the name of the **anterior elastic lamina,** or **the membrane of Bowman.** It cannot, however, be separated from the deeper part of the cornea, and it seems doubtful whether it differs from this except in not containing any cells. It is thicker and better developed in man than in most animals. Passing from its deep surface are bundles of fibres which traverse the subjacent lamellæ obliquely. They are called **fibræ arcuatæ ;** they seem to be a rudiment of fibres which in some animals (plagiostomatous fishes) pass perpendicularly through the corneal lamellæ, and pin them together (Ranvier).

On the posterior surface of the cornea is a layer which unquestionably differs both in structure and origin from the remainder. It is homogeneous, and when separated it curls up and breaks with a sharp fracture. It contains no cells, but can be decomposed into a number of very thin lamellæ. It is called the **posterior elastic lamina,** or **membrane of Descemet**. It is covered by a single layer of flat polygonal cells in every way resembling an endothelium, and which forms the anterior boundary of the anterior chamber of the eye.

There are no **blood-vessels** in the cornea.

There are also no true **lymphatic vessels**. The lymph circulates through the juice canals, which at the margin of the cornea open into the conjunctival lymphatic vessels. Some of the juice canals appear, however, to communicate with the channels in which the nerves run, and which are lined by an endothelium. It is possible that by these paths some of the lymph may be carried off.

The **nerves** are derived from the ciliary nerves, and enter in several bundles all round the edge of the cornea, having previously formed a plexus at the corneo-scleral junction. They branch freely after entering the cornea, and at some distance below the anterior surface they form a plexus with wide meshes: this is called the fundamental plexus. The nodal points of the nervous network are formed of a beautiful interlacement of fibrils. From this plexus branches come off, which run towards the surface of the cornea, and, since they pierce the anterior elastic lamella, are called perforating fibres. Having reached the anterior surface of the cornea proper, they give off lashes of fine fibrils, which run immediately beneath the epithelium, converging towards the centre of the cornea, anastomosing together, and forming a very close and fine plexus, called the sub-epithelial plexus. From this, fine fibrils come off and pass into the epithelium, where they ramify between the cells, and form a plexus called the intra-epithelial plexus. The final termination of the fibres is generally described to be by swollen knob-like ends beneath the surface of the epithelium; but Klein has discovered a still finer network than any hitherto seen, among the superficial cells, and this he looks on as the true termination of the nerves.

In the proper cornea the nerves terminate also by a network of very minute fibrils. There is no continuity between the nerves and the cornea corpuscles.

The nerves of the cornea, in consequence of the ease with which they can be shown by the gold method, have been for many years a favourite object of histological study, but it would be impossible here to describe all the details of their arrangement, for which we refer chiefly to the works of Klein and of Ranvier.

V. **The sclerotic** is formed of bundles of white fibrous tissue densely felted together. The arrangement in lamellæ is not very distinct, although most of the bundles run, in a general way, parallel to the surface. Posteriorly, corresponding to the fovea centralis of the retina, the tissue of the sclerotic is traversed perpendicularly or obliquely by a fibrous cord which interrupts the regularity of its tissue, and projects slightly at the posterior pole of the eye. This is supposed by Hannover to be a vestige of the closure of the eye-vesicle in fœtal life, and is called by him the **funiculus scleræ;** but according to Schwalbe it is formed merely by bundles of connective tissue accompanying some of the posterior short ciliary arteries, and does not correspond to the fovea centralis, and has no developmental importance. Mixed with the white fibrous tissue of the sclerotic are elastic fibres, and connective tissue cells lie between the fibrous bundles. Close to the corneo-scleral junction is a canal encircling the margin of the cornea. This is called the **canal of Schlemm.** It is lined by endothelium and contains a venous plexus, which communicates with the anterior ciliary veins. These can be injected from the anterior chamber of the eye, the injection passing through the spaces of Fontana and the canal of Schlemm, which, like the Pacchionian bodies of the membranes of the brain, establish a communication between the lymphatic and blood-vessels (Schwalbe). The inner boundary of the canal of Schlemm is formed of a peculiar tissue composed of elastic plates and networks covered by endothelioid cells. It is closely connected posteriorly with the ciliary muscle, and belongs really not to the sclerotic-cornea, or outer fibrous coat of the eye, but to the choroid-iris, or inner vascular coat, from which also Descemet's membrane and its endothelium are derived. The sclerotic is abundantly supplied with blood-vessels and nerves.

At the corneo-scleral junction the bundles of the sclerotic become continuous with those of the cornea. The sclerotic overhangs the cornea, so that the latter is set in the former somewhat as a watch-glass is in its frame.

VI. **Choroid and Iris.**—The outer fibrous coat of the eye is formed by the sclerotic and cornea, and is analogous to the dura mater of the brain. The second or vascular tunic, which corresponds to the pia mater and arachnoid of the nerve centres, is formed by the choroid and iris. This coat is deficient in front where the pupillary opening exists. It is closely attached to the sclerotic at the entrance of the optic nerve posteriorly, and in front at the corneo-scleral junction, where the attachment is effected by the laminated tissue which has been described as forming the inner boundary of the canal of Schlemm. Elsewhere the connexion between the sclerotic and choroid is very loose, and is effected by a tissue called the **membrana suprachoroidea.** This consists of membranes formed mainly of an elastic network covered by endothelial cells. Numerous flattened pigmented cells also lie on these membranes, and are sometimes branched, sometimes arranged edge to edge, like an endothelium. There are five or six membranes of this kind superposed on one another, leaving between them large spaces, which can be easily injected, and belong to the lymphatic spaces of the eye. The blood-vessels and nerves passing to the deeper parts of the eye traverse the m. suprachoroidea but this membrane has no vessels or nerves belonging to itself. When the choroid is torn away from the sclerotic, part of the pigmented tissue of the suprachoroidea remains attached to the inner surface of the sclerotic, and is called the **lamina fusca.**

Interior to the suprachoroidea is the **proper tissue of the choroid,** which consists mainly of blood-vessels held together and supported by elastic membranes with pigmented, mostly branched, cells, and covered by endothelium. These membranes are much more numerous and more closely placed than was the case in the suprachoroidea, so that the tissue is much firmer. The arrangement of the blood-vessels is peculiar, as the larger arteries and veins are contained in the outer part of the membrane, while the capillaries form a very dense plexus on the inner surface. The former layer is called the **stratum vasculosum** ; the latter the **chorio-capillaris.** The blood reaches the choroid chiefly by the short or posterior ciliary arteries. The long ciliary arteries run between the sclerotic and choroid, to the anterior part of the latter, and help to supply the ciliary processes and ciliary muscle, and form a circle about the outer margin of the iris, which is called the **circulus iridis major.** At the anterior part of the eye the sclerotic is perforated by the anterior ciliary arteries, which take part with the long ciliary arteries in the supply of the ciliary region and the iris, and form around the inner margin of the latter a vascular circle, the **circulus iridis minor.** The venous blood from the anterior part of the choroid leaves the eye in front by the anterior ciliary veins, which communicate with the venous plexus in the canal of Schlemm. All the blood from the iris, however, passes back, and enters the venous plexuses which form the bulk of the ciliary processes, and which also receive blood from the ciliary muscle and anterior part of the choroid. From the ciliary processes, veins run backwards, and collect the blood from the capillary coat of the choroid into four or sometimes five large trunks called **vasa vorticosa,** which perforate the sclerotic obliquely about the equator of the globe of the eye.

Between the stratum vasculosum and the chorio-capillaris is a thin layer which is free from pigment, and which contains the finest arteries and veins. There is here also an endothelial membrane. The veins of the choroid are surrounded by perivascular lymphatic spaces, whose outer wall is continuous with this membrane. In the chorio-capillaris are neither pigment cells nor elastic membranes, but the interspaces between the capillaries are filled by a homogeneous material of soft consistence, in which are some wandering cells, and which seems to be permeated by lymph (Sattler).

The capillary plexus of the chorio-capillaris is exceedingly close over all that part of the choroid which corresponds to the functional portion of the retina, but at the ora serrata it becomes much less dense, and is continued forward to the ciliary processes as a network, with elongated meshes. In this part, which is known as the **orbiculus ciliaris,** the endothelial membrane external to the chorio-capillaris is wanting, and the choroid contains much fibrous connective tissue.

Most internally the choroid is bounded by a homogeneous, structureless membrane, known as the **vitreous layer** of the choroid.

The **nerves** of the choroid are numerous, and are derived from the short ciliary nerves. They form a plexus in the supra-choroidea, in which are numerous ganglionic cells. From this come off non-medullated fibres, which supply the walls of the blood-vessels.

The iris consists of two portions—an anterior layer, continuous with the choroid, and a posterior layer, which is really a continuation forwards of the retina, although in a greatly simplified form. Both layers extend to the margin of the pupil.

The **anterior or connective-tissue portion** is composed of—1st, a layer of endothelial cells, continuous with those which line the posterior surface of the cornea and the trabeculæ of the ligamentum pectinatum iridis. 2nd, A layer of connective tissue, somewhat resembling the adenoid tissue of the lymphatic glands, consisting of spindle-shaped and stellate cells anastomosing and forming a reticulum, in whose meshes lymph corpuscles are found. In persons of dark complexion the cells contain pigment granules. 3rd, A layer of very loose connective tissue, in which are placed the chief blood-vessels and nerves of the iris and the muscular tissue. This latter consists of a ring of smooth muscular fibres at the pupillary margin (sphincter pupillæ), and a much less developed system of radiating fibres (dilator pupillæ). 4th, A very thin vitreous membrane, which has a fine radial striation. The nuclei of the muscular fibres in front, and of the anterior layer of the retinal portion of the iris behind, adhere closely to this vitreous layer, which has been looked on by some authors as muscular, and as constituting a dilator of the pupil. Its true nature has, however, been shown by Gruenhagen and Schwalbe.

The **retinal portion** of the iris consists of two layers. 1st, An anterior, continuous with the pigmented epithelium of the retina. It consists here of spindle-shaped, pigmented cells, closely adherent to the posterior surface of the vitreous layer, and radially placed, except near the pupillary margin, where they are circularly disposed. 2nd, A layer of polygonal-shaped cells, so deeply pigmented, that their nuclei and boundaries are difficult to see. Finally, a very thin cuticulum forms the posterior boundary of the iris. In blue irides the choroidal portion is free from pigment. In brown and black irides there is pigment in the cells of the second and third layers of the anterior portion.

The **vessels** of the iris have been already described.

The **nerves** lie chiefly in the third layer, and are for the supply of the muscular tissue of the iris itself and of the coats of the blood-vessels. There are no ganglionic cells in the iris, but in the nervous plexus which exists in the ciliary processes ganglionic cells have been found (Gruenhagen). The spaces between the cells and bundles of the second and third layers are of a **lymphatic** nature. They communicate through the spaces in the ligamentum pectinatum iridis with the anterior chamber. The blood-vessels of the iris are surrounded by lymphatic sheaths.

As has already been stated, there is a close connexion between the fibrous and vascular coats of the eye at the corneo-scleral junction, effected by the peculiar laminated tissue which has been described as forming the inner wall of the canal of Schlemm. This tissue is continued for a short distance between the proper corneal substance and the membrane of Descemet. To its outer or posterior part there is attached a quantity of smooth muscular tissue, which constitutes the **ciliary muscle.** This forms, on meridional section, a triangular-shaped mass. The outermost fibres, which arise in part from the sclerotic posterior to the canal of Schlemm, run backwards, and are inserted into the outer surface of the choroid as far back as the equator of the globe. More internally are fibres which run more radially—that is, in a direction towards the centre of the globe—and are inserted into the more anterior part of the choroid and outer aspect of its ciliary portion; while most internally are bundles of fibres which run a circular course, parallel to the margin of the lens (muscle of Müller). The nerves of the ciliary muscle are derived from the ciliary nerves. They form a plexus, with numerous ganglionic cells.

Filling up the angle between the outer margins of the cornea and iris, and forming the outer boundary of the anterior chamber, is a peculiar structure, known as the **ligamentum pectinatum iridis.** This consists of bundles of fibres derived from the tissue which forms the inner wall of Schlemm's canal.

The bundles divide and unite with one another, so as to form a spongy tissue. They are covered by endothelium, which is continuous with that lining the posterior surface of the cornea, and in their outer or corneal portion have, under the endothelium, a continuation of the structureless membrane of Descemet. They are attached to the anterior surface of the iris. The spaces which are enclosed in the reticulated tissue of the the ligamentum pectinatum are called the **spaces of Fontana.** They communicate on the one hand with the anterior chamber, on the other with the canal of Schlemm, and through this with the anterior ciliary veins (Schwalbe).

VII. **The retina** forms the third or internal coat of the eye-ball. While the other coats are of connective-tissue origin, the retina is purely nervous. It is the first portion of the eye which is formed, and arises on each side as a spherical hollow outgrowth from that portion of the brain which will subsequently form the third ventricle. In its origin the retina is precisely analogous to one of the cerebral hemispheres. It is attached to the brain by a hollow, constricted stalk, the future optic nerve, through which the cavity of the brain communicates freely with that of the retina. In this stage it is called the **primary optic vesicle.**

That part of the primary optic vesicle which is next the surface of the body is soon pushed back into the cavity of the vesicle; and the result of this invagination is that the original cavity is obliterated, and the outer portion of the retina comes to lie close to the inner, and these two portions bound a cup-shaped cavity, which is open externally. In this stage the retina is called the **secondary optic vesicle.** The invagination does not take place exclusively from without, but also from below, so that at first the secondary optic vesicle has in its inferior part a fissure, through which there grow into its cavity the connective tissue and vessels which will subsequently form the vitreous body. This fissure, however, soon becomes obliterated by coalescence of its edges, and then the secondary optic vesicle is really cup-shaped, its open mouth, where the two layers forming its wall pass one into the other, being somewhat constricted, and occupied by the rudiment of the crystalline lens, whose origin from the epidermis will be subsequently described.

The two layers of the wall of the secondary optic vesicle, or, as they may now be called, of the retina, soon show marked differences; for while the outer layer, or that furthest from the centre of the eye-ball, remains thin, soon becomes pigmented, and ultimately develops into a single layer of flat epithelial cells, the inner layer, or that which was invaginated, increases greatly in thickness, remains free from pigment, and gives origin finally to a most complicated structure, consisting of nerve fibres, ganglionic cells, neuro-epithelium, supporting connective tissue, and blood-vessels.

The retina, thus constituted, has its elements arranged in layers lying one over the other. These **layers,** reckoned from within outwards, are the following:—

1. Internal limiting membrane,	Developed from internal layer of secondary optic vesicle.
2. Nerve fibres,	
3. Ganglionic cells,	
4. Internal reticular layer,	
5. Internal granules,	
6. External reticular layer,	
7. External granules,	
8. External limiting membrane,	
9. Rods and cones,	

10. Pigmented epithelium—developed from external layer of secondary optic vesicle.

Of these layers, 1 and 8 belong to the **supporting connective tissue.** This consists of fibres **(Müller's fibres),** which run radially, and traverse all the layers of the retina, except the pigmented epithelium. Each fibre expands at its inner end into a conical-shaped enlargement, which frequently contains a nucleus. The bases of these enlargements, which are turned inwards, and lie internal to all the other parts of the retina, meet by their edges, and form the **internal limiting membrane,** which is thus, not a separate membrane, as was formerly supposed, but merely the expanded inner ends of Müller's fibres, which are here strengthened by a homogeneous cuticulum, most developed at the peripheral portion of each conical expansion. Müller's fibres, as they pass through the retina, give off fibrous and membranous processes, which support the nervous elements of the different layers. Externally the fibres again coalesce to form a thin membrane **(external limiting membrane),** which appears in sections as a fine, sharply-marked line. This membrane is perforated by innumerable holes, through which the rods and cones pass. From the outer aspect of the membrane fine threads extend between the rods and cones, and support these bodies. Each fibre of Müller, in that part of it which passes through the layer of internal granules, has an oval nucleus, an indication of its originally cellular structure. In chemical composition the inner portion of Müller's fibres is soft and albuminous, while that which lies in the outer layers of the retina is firmer and more horny.

A distinction has recently been made among the layers of the retina, which greatly simplifies the conception of this difficult organ. It has been shown by Schwalbe, W. Müller, and others, that, while layers 1 to 6, consisting, as they do, of nerve fibres, ganglionic cells, and connective tissue, resemble a portion of the brain, 7 and 9 are of an epithelial nature. Each rod and cone is connected with an external granule, which is, in fact, its nucleus; and the compound bodies thus formed are, in most respects, identical with the modified epithelial cells which form the end organs of the nerves in the other organs of special sense. It will be observed that those parts which immediately bound the cavity of the primary optic vesicle develop into epithelium, just as do those parts which bound the ventricles of the brain and the central canal of the spinal cord. In the retina, as elsewhere, blood-vessels do not penetrate into epithelium ; consequently, we do not find any vessels external to the layer of internal granules.

A, necessarily, very short description will now be given of the different layers of the retina.

1. The **internal limiting membrane** has been already described.

2. The **nerve fibres** are continuous with those of the optic nerve. They lose their white substance as they pass into the eye through the lamina cribrosa, and spread out over the surface of the retina as fine, naked axis cylinders, commonly presenting varicose swellings in their course. They run in bundles, which have, on their exterior, flat connective-tissue cells, similar to those of the neuroglia of the brain (Schwalbe). The layer of nerve fibres is necessarily thickest near the optic entrance, and thins out towards the periphery of the retina.

3. The **ganglionic cells,** in most parts of the retina, form a layer only one cell deep. Each cell is multipolar. One unbranched process passes into the layer of nerve fibres, with one of which it becomes continuous. It is identical with the axis cylinder process of the ganglionic cells of the brain and cord. The other processes branch freely, and pass, more or less radially, into the inner reticular layer, where they are lost to view. Their termination is not certainly known, but they probably are continuous with the cells of the layer of internal granules. Between the ganglionic cells are flat connective-tissue corpuscles and a soft, semi-fluid, cementing substance.

4. The **inner reticular layer** has a granular appearance, but is really composed of an extremely fine reticulum of fibres, which are formed of a horny material, and belong to the supporting connective tissue of the retina. It is traversed by processes of

the ganglionic cells and of the inner granules; also by the radial fibres of Müller, which, however, have no connexion with the reticulum. There are no nuclei or cellular structures in the inner reticular layer.

5. The **inner granule layer** consists, for the most part, of minute, oval, bipolar nerve-cells (granules). Each cell sends a fine, varicose, unbranched process into the inner reticular layer, where it probably becomes continuous with the processes of the ganglionic cells, or with one of the nerve fibres of the second layer, although these connexions have not been certainly demonstrated. From the outer end of each granule there passes into the external reticular layer a thicker process, which branches freely in a horizontal direction, and has been shown to be continuous with the internal prolongations of the cones (Gunn), and possibly of the rods.

Besides the bipolar granules, which are genuine nerve-cells, there are, in the inner portion of this layer, other cells, which are larger, and which stain more deeply, and have each but one process, which passes into the inner reticular layer, with whose formation these cells are supposed to be concerned. As has been already mentioned, each fibre of Müller, as it traverses the layer of inner granules, possesses an oval nucleus.

6. The **external reticular layer** resembles the fourth layer in being formed of a very close and fine reticulum. It differs, however, in possessing numerous cells, which are flattened in the plane of the retina, in which plane they branch freely. A fine plexus of nerve fibres, derived from the outer processes of the inner granules, also occurs in this layer.

7 and 9. The **rods and cones**, with their internal prolongations, in which the **internal granules** are placed, are modified epithelial cells. Each of them passes through a perforation in the **external limiting membrane,** and is thus naturally divided into

two parts—an external, lying outside this membrane; and an internal, lying between it and the external reticular layer. The external part consists further of two segments—an outer and an inner.

Of these epithelial cells there are two chief varieties, which are called respectively rods and cones.

The outer portion of the **rods** consists, as said, of two segments. The **outer segment** is an elongated cylindrical body, of a shining appearance. It blackens in osmic acid, and does not stain in ordinary dyes, such as carmine, logwood, &c. It presents a fine longitudinal striation, due to shallow grooves on its surface, and a transverse striation, due to its being composed of a number of plates joined to one another by cementing substance. This laminated structure is enclosed in a fine sheath, structureless, and of a horny consistence. The **inner segment** is granular and protoplasmic, stains feebly in carmine, and does not blacken in osmic acid. It is cylindrical or slightly swollen, and of somewhat greater diameter than the outer segment. The line of junction with the latter is transverse and distinct. Just within this the inner segment contains a plano-convex body, known as the **ellipsoid,** which in the human retina has a fibrous structure. The inner segments of the rods, as well as of the cones, are supported by fine thread-like processes from the outer surface of the external limiting membrane.

After its passage through this membrane the inner segment of each rod passes into a very fine varicose thread **(rod fibre),** which is continued inwards to the external reticular layer, at whose external limit it appears to terminate in a minute spherical or oval enlargement. At one point the thread is swollen by a nucleus, which is contained within it. This is the nucleus of the entire modified cell, and is commonly spoken of as an **external granule.** It is peculiar in presenting a transverse striation, formed by alternate layers of a bright and dark substance. The position of the nucleus is in some cases close to the external limiting membrane; in others at different distances further in, closer to the external reticular layer. Consequently, the granules, or nuclei, lie in several layers, one above the other, and form a stratum of considerable thickness. The thinner and more numerous the rods, the more numerous are the granules, and the thicker the layer which contains them.

The **cones** consist of the same parts as those which form the rods. The **external segment** is shorter than that of the rods, and is conical, with the apex externally. It consists of a horny sheath, containing a laminated structure, formed of plates held together by cementing substance. The **inner segment** is much thicker than the corresponding part of the rods. It has in men a large elipsoid, which has a fibrous structure (Schultze). In many animals the outer end of the inner segment is occupied by a fatty globule, which in birds and reptiles is coloured red, green, yellow, or sometimes blue. In all vertebrates, except mammals, double cones are found, which are free at their outer end, but joined together at their inner extremity.

After its passage through the external limiting membrane the inner segment of the cone passes into a **fibre,** which is much thicker than the rod fibre, and which presents a longitudinal striation. It traverses the layer of external granules, and terminates at the external reticular layer in a conical expansion, from the base of which fine fibrils come off, and enter the reticular layer, running a horizontal course. In each cone fibre there is a nucleus: this is oval in shape, and situated immediately internal to the limiting membrane. It has not the striation of the rod nuclei, but possesses a nucleolus. In most parts of the human retina the cones are much fewer than the rods, three or four of the latter being found in the line separating one cone from the next. In some animals the cones are much more numerous than the rods, or are exclusively present (reptiles);

while in others the cones are very few, or altogether absent (animals of nocturnal habits).

10. **The epithelium,** developed from the external layer of the secondary optic vesicle, consists of a single layer of flat cells, which are for the most part of a hexagonal shape. The outer part of each cell is free from pigment, and is bounded externally by a horny cuticulum. The non-pigmented portion of the cell contains in many animals fat globules, sometimes of a yellow colour, and masses of a substance similar to that which forms the external segments of the rods and cones. The inner portion of the cell contains black or dark-brown pigment, occurring as minute, elongated, crystalline masses. At the junction between the two portions is situated a round or oval nucleus. The outermost portions of the rods are embedded in depressions in the inner pigmented portion of the epithelial cells. These cells send numerous thread-like processes inwards between the outer segments of the rods and cones. The processes are seen sometimes to contain pigment, and sometimes to want it; and the remarkable fact has been ascertained, that when the eye is exposed to light the pigment passes down in the protoplasmic processes of the epithelial cells, so as to lie in quantity between the rods and cones, while in darkness it retracts, so as to lie altogether external to these bodies. In Albinos the pigment is altogether wanting in the epithelial cells.

Where the optic nerve enters the eye, the layers of the retina are absent, and this part is called the **optic papilla**. Since the fibres of the nerve diverge in all directions, they leave in the centre of the papilla a hollow, with sloping edges. This is called the **physiological excavation,** to distinguish it from the excavation with abrupt or overhanging edges, which is common in cases of glaucoma.

External and slightly inferior to the optic papilla is a portion of the retina, which presents many remarkable structural peculiarities. This part is called the **macula lutea,** because here all the layers of the retina, as far out as the external reticular layer, are diffusely stained of a yellow colour. At the peripheral parts of the macula the retina is somewhat increased in thickness, but becomes gradually thinner towards the centre, where for a certain extent all the inner layers are deficient, and with them the yellow pigment and blood-vessels. This thin part is called the **fovea centralis.**

The optic nerve-fibres bend around the macula lutea, and, becoming gradually fewer, cease at some distance from the fovea centralis. The layer of ganglionic cells is increased in thickness; the cells are bipolar in shape, obliquely placed, and form no longer a single row, but lie over one another, seven or eight deep. They extend further inwards than the layer of nerve fibres, but gradually disappear before reaching the deepest part of the fovea. The layer of internal granules also becomes thinner, and vanishes. The two reticular layers coalesce, and, becoming very thin, extend in a rudimentary condition over the fovea. At the peripheral parts of the macula lutea the rods become very few, while the cones increase in number, and further inwards cones only are found. These are much thinner and longer than in the other parts of the retina. In consequence of their great number, there is not room for all the cone granules or nuclei to lie in a single row under the external limiting membrane, but they are placed in several rows, as close as may be to this. The cone fibres no longer run perpendicularly to the plane of the retina, but diverge from the fovea centralis, forming a layer, which has been named the **external fibrous layer of the retina** (Henle). In the middle of the fovea the retina consists only of the pigmented epithelium, the cones with their granules and diverging fibres, and the rudimentary reticular layers.

At some distance behind the ciliary processes the retina, as seen by the naked eye, appears to terminate abruptly with a jagged margin, which is called the **ora serrata**. Up to this line the thickness of the retina diminishes only slightly, but here all the layers cease rather suddenly, except the pigmented epithelium, which is continued over the inner surface of the ciliary processes and the back of the iris. Internal to this is a single layer of columnar-shaped cells, which have no pigment where they cover the ciliary processes, but are pigmented on the iris. These represent the inner layer of the secondary optic vesicle. Internal to them is a structureless cuticular membrane. The retina thus simplified is called the **pars ciliaris retinæ** where it covers the ciliary processes and the choroid between these and the ora serrata, and the **pars iridica retinæ** where it lies on the back of the iris.

In the living eye the outer segments of the rods have a purple colour. The pigment can be dissolved in bile and other fluids. It fades very rapidly on exposure to the light, but is reproduced so long as the choroidal tissues and the external epithelium of the retina retain their vitality. It has been exhaustively studied by Kühne. It is called **rhodopsin** or **visual purple.** In some animals a few rods are coloured green. The visual purple is wanting in those parts where rods are absent, viz.: the macula lutea, and for a short distance behind the ora serrata.

The **blood-vessels of the retina** are derived from the central artery, and, except at the entrance of the optic nerve, have no connexion with the ciliary vessels. The arteries are all 'terminal,' that is, they communicate with one another only by capillaries. The larger vessels lie in the layer of nerve fibres, while the capillaries extend out as far as the inner granule layer. It is said that in the eel there are vessels in the external granule layer (Denissenko). This is the only instance in which vessels have been observed to extend into the epithelial portion of the retina, and this observation is not undisputed.

Around the retinal vessels are perivascular lymphatic spaces which can be injected by puncture from the pial sheath of the optic nerve.

The **optic nerve** consists of fine nerve fibres which resemble those of the brain and cord in possessing a sheath of myeline, while they want a neurilemma. The fibres are arranged in bundles, which are separated by connective-tissue septa derived from the pial sheath of the nerve, and which contain blood-vessels derived from the ciliary arteries. Connecting the nerve fibres is a soft connective tissue (neuroglia) with flat cells, which lie not only on the exterior of the bundles but also among the fibres. At about half an inch behind the eye the central artery and vein pierce the optic nerve, and, accompanied by connective tissue, run in its axis to the optic papilla, from which their branches spread out over the retina. The optic nerve is surrounded by sheaths derived from the brain membranes. The external is continuous with the dura mater; the internal closely surrounds the nerve and is continuous with the pia mater; between these is a space divided into two by a thin membrane derived from the arachnoid. The subdural space or that between the dura mater and arachnoid, and the subarachnoid space, or that between the arachnoid and pia mater, are traversed by numerous fibrous trabeculæ, and communicate with the corresponding spaces around the brain. The subarachnoid space also communicates freely with the suprachoroidal lymph space between the sclerotic and choroid coats of the eye.

At the back of the eye the sheaths of the optic nerve become continuous with the ocular tunics; the pial sheath passes in part into the choroid, but chiefly into the sclerotic, with which the other sheaths are continuous.

As the optic nerve passes through the coats of the eye it becomes much narrowed in consequence of the nerve fibres losing their myeline. The bundles of nonmedullated nerve fibres here traverse a close network of connective tissue derived from the sclerotic and choroid, and called the **lamina cribrosa.** At this place there is a communication in the nerve between the central artery and the ciliary arteries.

VIII. The **crystalline lens** is an epidermic structure, and is originally formed as a depression of the general surface of the head, from which it is soon completely separated. It is at first a hollow vesicle lined by epidermic cells. The posterior wall of this vesicle increases in thickness and encroaches on the cavity, which is finally obliterated.

The mature lens is enclosed in a capsule, which is thicker on the anterior than on the posterior surface. The capsule is very elastic, but differs from both elastic and ordinary connective tissue. It resembles the sarcolemma of muscle, and the basement membrane of gland-vesicles (Schwalbe). It has no cells or nuclei or vessels, and presents an obscurely lamellar structure. It is partly derived from the epidermic cells of the lens as a cuticular formation, partly from a vascular membrane which covers the lens in fœtal life (Schwalbe).

Underneath the capsule of the anterior surface of the lens is a single layer of flat polygonal cells. At the edge or equator of the lens these pass gradually into elongated fibres of which the mass of the lens is composed. The fibres are the modified epithelial cells of the posterior wall of the original lens vesicle. Each is hexagonal in cross section, but so that two opposite surfaces are much longer than the others. In this way each fibre is a flattened band. Except in the most central parts of the lens each fibre contains one nucleus. The outer portion of the fibre is denser than the central parts, but there is no separable sheath or cell-wall. From the surface short spines project, which help to unite neighbouring fibres, as the cells of the stratum spinosum of the epidermis are joined. The fibres are also joined together by cementing substance into lamellæ, which are arranged parallel to the surfaces of the lens. In a macerated and hardened lens a stellated figure is seen on the anterior, and another on the posterior surface. These are accumulations of cement in which the fibres terminate. The lens has neither blood-vessels nor nerves.

IX. The **vitreous body** is a transparent semifluid mass which fills the space between the lens and the back of the eye. It is enclosed in a structureless capsule named the **hyaloid membrane.** This lies in apposition with the internal limiting membrane of the retina. Immediately internal to the hyaloid membrane are scattered cells resembling white blood corpuscles, and which have probably emigrated from the vessels of the adjacent parts.

The vitreous body itself contains 98.6 per cent. of water, which holds in solution salts, albumin, and mucin. Through the centre of the vitreous body there extends a canal reaching from the optic disc to the posterior surface of the lens. It is bounded by a membrane, and contains fluid similar to that which forms the bulk of the vitreous. In fœtal life this canal contained an artery (hyaloid artery) derived from the central artery of the retina, and destined for the supply of the lens. At the peripheral part of the vitreous body, and parallel to its surface, fissures exist filled with fluid. These are not bounded by membranous walls, but by the substance of the vitreous body itself. In the vitreous body very few fibres exist except in front, where they lie near the surface, and help to form the zonula of Zinn. Numerous cells are found in the vitreous body. They present various shapes: many of them are branched, and they may contain several nuclei and also vacuoles filled with clear fluid. They are all wandering cells which have reached the vitreous from without. The vitreous body, which in fœtal life is formed of connective tissue, has, in the mature eye, undergone such modification that all the fixed cells and most of the fibres have disappeared, while there remains only the albuminous cementing substance infiltrated with water, and permeated by wandering cells (Schwalbe).

Anterior to the ora serrata the hyaloid membrane loses its homogeneous structure, and presents stiff, shining fibres, which run forwards in contact with the pars ciliaris retinæ. This fibrous portion of the hyaloid membrane is thrown into folds, which are at first very shallow, but at the ciliary processes become very prominent, and dovetail with these projections of the choroid. The summits of the ciliary processes are in contact with the deepest part of the depressions between the folds of the hyaloid membrane, whereas the summits of the folds of the latter do not reach the deepest part of the valleys between the ciliary processes. Consequently, corresponding to each of these is a space, which opens in front into the posterior chamber of the eye. These spaces are the **recessus cameræ posterioris** (Kuhnt). From the apices of the ciliary processes the hyaloid membrane runs a free course to the edge of the lens, and in this part forms part of the boundary of the posterior chamber of the eye. Here the fibres are collected into bundles, which are separated from one another by fine fissures. Of the bundles, those which correspond to a valley between two ciliary processes pass to the anterior surface of the lens, and become continuous with the exterior of its capsule; while those bundles which correspond to the summit of a ciliary process pass to the edge of the lens, and extend a slight distance on its posterior surface, where they also become continuous with the capsule. All that part of the hyaloid membrane which has a fibrous structure and is thrown into folds—that is, that part which extends from the ora serrata to the edge of the lens—is called the **zonula ciliaris,** or the **zonula of Zinn.**

Behind the anterior part of the zonula, and surrounding the edge of the lens, is a space which, on meridional section of the eye, appears of a triangular shape. This is known as the **canal of**

Petit. It is bounded in front by the zonula, internally by the edge of the lens, and posteriorly by the vitreous body. It is commonly stated that the hyaloid membrane divides in front into two layers, of which the posterior separates the canal of Petit from the vitreous body; Schwalbe, however, holds that there is no membranous posterior wall to Petit's canal, but that this is bounded merely by the naked vitreous body. He further finds that the canal of Petit communicates freely with the posterior chamber of the eye by means of the fissures between the bundles of the free portion of the zonula of Zinn.

1. **The eyelids** may be hardened in alcohol alone, or chromic acid, one-sixth per cent., for a week, and then in alcohol.

Sections made vertically and at right angles to the surface. Logwood or picrocarmine; balsam.

The structures described above will be easily recognised.

2. **Lachrymal gland** hardened in alcohol. Logwood; balsam.

Appearances similar to those seen in serous salivary glands.

3. **Cornea** of dog, cat, rabbit, &c. Hardened in Müller's fluid or chromic acid, one-sixth per cent.; then alcohol. Vertical section. Logwood or picrocarmine; glycerine or balsam.

The different layers will be seen. The cells will appear as narrow bodies lying between the lamellæ.

4. Macerate a fresh cornea for twenty-four to forty-eight hours in Müller's fluid diluted with an equal bulk of water, or in dilute alcohol. Scrape the anterior surface with a sharp scalpel, and diffuse the scrapings in a drop of picrocarmine; cover, and place in the moist chamber until the cells are stained; then allow glycerine to run in under the cover-glass. The cells will be got isolated, and their various shapes will be well seen.

5. Remove carefully the cornea of a recently-killed rabbit, taking care that the membrane is not dragged, or soiled with blood or hairs. Place at once in freshly-expressed lemon juice, and leave for five minutes. Wash rapidly in distilled water, and place in chloride of gold (one per cent. solution in distilled water); put it away in the dark, and leave for twenty minutes. Then wash again in distilled water, and place in water to each ounce of which one drop of acetic acid has been added, and expose to the light. In a short time the large nerve trunks will appear as dark lines, and after some hours or days, according to the light and temperature, the whole cornea will have assumed a purple or dark-blue appearance. It is then placed for some

time in alcohol, which completes the hardening, and also prevents the gold staining from becoming diffuse (Ranvier).

Make vertical and horizontal sections, and mount in glycerine.

In the vertical sections the cornea corpuscles will appear as darkly-stained linear bodies between the lamellæ, as in 3. Portions of nerves will be seen stained black, the larger trunks in the mid-depth, the smaller near the anterior surface. Just under the epithelium, fine varicose threads may be seen to run, and from these, or directly from deeper fibres, minute fibrils may be seen to ascend into the epithelium, and ramify among the epithelial cells.

In horizonal sections the branching cornea corpuscles will be seen from their surface, and appear as stellate anastomosing figures. (They may be even better seen by tearing a gold cornea into lamellæ with fine forceps.) The different nerve plexuses will be seen, according to the depth of the section from the anterior surface.

6. The eye of a recently-killed frog is enucleated, washed in distilled water, and placed in three per cent. nitrate of silver solution. In a few minutes it is removed, and the epithelium gently scraped off the cornea. The eye is replaced in the silver solution for twenty minutes. It is then removed, and washed in distilled water, in which it is exposed to sunlight, with the cornea uppermost, until the latter assumes a deep brown colour. The cornea is then very carefully removed with fine scissors. Three or four radial notches are made in its edge, so that it will lie flat, and it is mounted in glycerine. If it be mounted without removing the posterior endothelium, this will be seen, the outlines of the cells being made evident by the silver. These and the nuclei have often very irregular contours. The endothelium may be removed by brushing with a camel's hair pencil, and also by the same means any remnants of the anterior epithelium. The basis-substance of the cornea will then be seen of a dark-brown colour. On this are white branching and anastomosing figures, which are the spaces in which the cornea corpuscles lie. Traversing the cornea are branching, clear canals, lined by elongated endothelial cells, whose outlines appear as fine dark lines. These are the channels in which the larger nerves run. It may be seen that some of the branching spaces communicate with these canals. If such a cornea is stained in logwood, the nuclei of the cornea corpuscles will appear of a blue colour in the interior of the clear spaces.

The cornea may be stained with silver by scraping off the epithelium and rubbing the surface with a solid stick of nitrate of silver. The cornea is then removed and mounted in glycerine, and exposed to the light until it becomes brown. In some animals the cornea corpuscles lie in groups and the cells meet by flat edges—like an endothelium. The spaces which contain these groups communicate by broad channels. This arrangement can be well seen in the cornea of the cat when it has been stained with silver.

8. The eye of a rabbit is divided with a sharp razor into an anterior and a posterior half. The former is hardened in Müller's fluid for a fortnight, then spirit. Or in chromic acid and spirit. The posterior capsule of the lens is then opened and the lens removed. With sharp scissors a radial segment is cut out, starting from the centre of the cornea and including all the coats—cornea, sclerotic, iris, choroid, ciliary processes, and ciliary muscle. This piece should be stained *en masse* in logwood, then dehydrated, and then soaked in oil of cloves. It should then be placed in cacao butter just melted, and kept in this for an hour or two until it is completely infiltrated with the mass. It is finally embedded in the cacao butter, using a paper box, as was done when the wax and oil mass was employed. The object should be so placed in the box that the sections may have a radial direction from the centre of the cornea. The sections, which are best made with a microtome, should be placed for some minutes in oil of cloves, which dissolves out the cacao butter, and then mounted in balsam (Schäfer). Instead of cacao butter paraffiin may be used: the details of this process will be given in the appendix. If successful, these sections show the parts which have been noticed as occurring at the corneo-scleral junction and the adjacent structures, which will be readily recognised, and need not be again described.

9. If from another portion of the same eye the sclerotic and **choroid** be separated, and some of the loose tissue extending between them be teased in a drop of water or glycerine, the flat branching connective-tissue cells will be seen, filled with black or yellowish-brown pigment. In the dog the cells are not branched, but are flat plates marked with lines where the pigment is deficient, and which are impressions of elastic fibres on which the cells lie.

Vertical sections through the choroid, hardened in Müller's fluid and alcohol, show the pigmented connective tissue and the numerous blood-vessels. The larger ones external, the capillaries near the inner surface.

On the inner surface of the choroid the flat hexagonal retinal epithelium will be found, and can be removed by gentle scraping.

10. **The lens** contained in its capsule is hardened for a fortnight in Müller's fluid, then in alcohol. Sections are made, stained in logwood and mounted in glycerine. These will show the fibres and epithelium in situ. Portions may be torn up in glycerine or water, when the isolated fibres will be seen. It will be observed that epithelium exists only on the anterior surface of the lens, lining the capsule. When the fibres are seen in cross section they appear as hexagonal figures, of which two opposite sides are longer than the other four. In the isolated fibres the serrated margins will be seen, more distinct in some

animals, as frogs, than in mammals. The anterior epithelium may be shown also by staining the fresh lens in nitrate of silver, and shaving off a thin layer from the anterior surface. The transition of the epithelial cells into the lens fibres at the equator is best seen in small lenses, such as that of the frog.

11. To harden the **retina** it is better not to open the eye, at least for the first few hours of the hardening. If the fresh eye be opened the retina is apt to fall into folds, and separate from the choroid. The eye must be quite fresh : two or more short cuts should be made with a razor through the coats, and the eye suspended in Müller's fluid, or a mixture of chromic acid and spirit. In two or three days the globe may be divided across, and the posterior half again placed in the hardening fluid. The hardening is completed in the usual way by alcohol. Portions of the retina are then carefully cut out, stained with logwood or picrocarmine, and embedded in cacao butter, after having been treated as in 8, or in paraffin. The sections must be very thin. They may be mounted in balsam, or, after dissolving out the cacao butter by oil of cloves, the sections may be placed in spirit (which removes the oil of cloves) and then mounted in glycerine.

The retina may be torn up in order to isolate its elements. It is best to use the frog's retina whose structure is simple. Portions of the fresh membrane should be placed for twenty-four hours in osmic acid, 1 per cent. Then in water to which a little glycerine is added. Small fragments may be torn up with needles in a drop of glycerine. The rods with their large external segments, and the small cones between, will be easily seen, and sets of these connected with their external granules will be met with, as well as fragments of the other layers. The retina is an extremely difficult object, and students will only waste their time in attempting to prepare it until they have acquired considerable dexterity in microscopic manipulation.

CHAPTER XVIII.

THE EAR.

I.—*The Tympanum.*

THE **membrana tympani** consists of three layers. The outer is a continuation of the skin of the meatus. It is extremely delicate, and the corium has no papillæ. The inner layer is part of the mucous membrane of the cavity of the tympanum. It is covered by a simple scaly epithelium. The middle layer is peculiar to the membrana; it consists of fibrous tissue, arranged in bundles, and of elastic fibres. The fibres and bundles have a circular direction in the inner part; externally they radiate from the handle of the malleus.

The membrane is freely supplied with blood-vessels, nerves, and lymphatics.

The **cavity of the tympanum** is lined by a very delicate mucous membrane, which is continued into the mastoid cells. It is covered in the lower part of the cavity by columnar ciliated epithelium, continued from the Eustachian tube; but over the membrana tympani, the ossicula, the upper part of cavity, and the promontory the epithelium is simple squamous. A gradual transition occurs from one kind of epithelium to the other. There are said to be mucous glands in the lining membrane of the human tympanum.

II.—*Vestibule and Semicircular Canals.*

The **utricle** and **saccule** and the **membranous semicircular canals** lie in the corresponding bony hollows, from whose inner surface they are separated by the perilymph. This fluid does not, however, completely surround the membranous labyrinth. The utricle and saccule lie close to the inner wall of the vestibule, and the membranous semicircular canals lie against the bone, on the convex aspect of the canals. In this position they are held by ligamentous bands, which pass to them from the periosteum lining the bony labyrinth. Branching bands also extend across the perilymphatic spaces from the periosteum to the free wall of the membranous canals. These extensions from the periosteum form a **fibrous** or **outer coat** for the membranous labyrinth. The periosteum, the ligaments, and the outer surface of the membranous labyrinth are covered by endothelium.

Under the fibrous coat is the **membrana propria,** or basement membrane, which is a homogeneous structure, thinner at the side which lies next to the bone than elsewhere. It possesses in man papilla-like prominences, and is lined by a single layer of **flat epithelium.**

Where the nerves enter the utricle and saccule the wall presents an internal prominence, called the **macula acustica,** and a corresponding prominence at the nerve entrance into each ampulla of the semicircular canals is called the **crista acustica**. At these points the membrana propria is thickened, and contains pigment cells. The nerve fibres, having formed a plexus, pierce the basement membrane, losing their white substance, and form a network of fine fibrils.

The **epithelium** here consists of two kinds of cells. In one, the cells are columnar, and have on their free ends long hair-like projections. Their deep or attached ends are continuous with nerve fibrils. These cells are an example of neuro-epithelium, and correspond to the rods and cones of the retina, with their respective granules and fibres. Between the columnar cells are others, which are long and thin; they rest by an expanded foot on the basement membrane, while their free ends are continuous with a membranous cuticulum, which is perforated by the hairs of the columnar neuro-epithelium. The second kind of cells have no connexion with the nerves, and serve probably as supporting organs for the columnar cells.

Resting on the maculæ and cristæ acusticæ are groups of minute crystals of carbonate of lime, enclosed in a gelatinous substance. They are called **otoliths.**

III.—*Cochlea.*

The **cochlea** is a tube coiled in a spiral around a central pillar of bone, called the **modiolus.** This tube is divided by a septum, extending from its inner side, or that next the modiolus, to its outer wall. In its inner part the septum is formed of a spiral crest of bone, projecting from the modiolus, and called the **lamina spiralis ossea;** and in its outer part by a tense membrane, called the **membrana basilaris,** which is attached internally to the lamina spiralis ossea, and at its outer part to a mass of connective tissue, having on cross section a somewhat triangular shape, and called the **ligamentum spirale.** The latter is a thickened and somewhat modified part of the periosteum of the bony cochlea.

On that surface of the lamina spiralis ossea which looks towards the apex of the cochlea the periosteum is greatly thickened, and forms a projection, with overhanging edge looking outwards. This is called the **crista spiralis**. From the upper surface of the thickening, there comes off a membrane, which passes upwards and outwards, and is attached to the upper part of the outer wall of the cochlear tube, at a considerable distance above the attachment of the membrana basilaris. This is the **membrane of Reissner.**

In this way the cochlear tube is divided longitudinally into three compartments—one lying below the membrana basilaris and lamina spiralis; this is the **scala tympani:** one above the membrane of Reissner; this is the **scala vestibuli;** and one, triangular on cross section, lying between the membrane of Reissner and the membrana basilaris: this is the **ductus cochlearis,** or **scala media**. This latter portion contains the organ of hearing, and is analogous to the membranous semicircular canals. The scalæ tympani and vestibuli are lymph spaces, and analogous to the other perilymphatic spaces of the labyrinth. The ductus cochlearis is closed above, and below it communicates with the saccule by a minute canal, called the **canalis reuniens.**

On the membrana basilaris is seated a highly-complicated epithelial structure, called the **organ of Corti.** In brief, it consists of two rows of rod-like bodies, which rest on the membrana basilaris at some distance from one another by one end, while by the other they are inclined towards each other, and meet in a sort of articulation. These rods are called the **pillars of Corti,** and they form, with the portion of membrana basilaris between their feet, a triangular-shaped space, running the entire length of the cochlea. This space is called the **tunnel.** The internal pillars are more numerous than the external. Each of the latter articulates with two inner pillars. In the upper end of each pillar is the vestige of a nucleus, and occupying the acute angle between the basilar membrane and the attached end of each pillar is a nucleated mass of granular protoplasm. At each side of the tunnel are disposed the cells which form the end organs of the auditory nerves. These are columnar-shaped cells, which carry on their free ends, fine hair-like processes. On the inner side of the tunnel (that is, next the modiolus) there is one row of such cells; on the outer three or four. Each of these outer cells is a compound body, formed of two cells—a pyramidal cell, which has the hairs on its larger free end, and, connected with it, a spindle-shaped cell. The former are the **cells of Corti ;** the latter the **cells of Deiters.** Beyond these sensory cells, both on the outer and inner sides of the tunnel, are epithelial cells, at first of a high columnar shape, but which diminish in length as they recede from the pillars of Corti. Covering the hair cells is a cuticular membrane, starting from the upper ends of the pillars of Corti, and becoming gradually lost external to the hair cells. It is perforated, to allow the free ends of the latter to project through it. It, consequently, is full of holes, and is called the **membrana reticularis.**

Attached to the crista spiralis, close to the inner attachment of Reissner's membrane, is a membranous structure, which extends outwards over the organ of Corti. It is thin at its origin, but becomes thicker externally, and is in close contact with the outer hair cells. It is called the **membrana tectoria.**

The **nerves** run in the axis of the modiolus, and all along its course bundles come off, and pass through the lamina spiralis ossea. In this, or in the modiolus internal to it, they meet a mass of bipolar ganglionic cells, which runs spirally all along the inner wall of the cochlea, and is called the **spiral ganglion.** It is probable that each nerve fibre has on its course one ganglionic cell. Beyond the ganglion the fibres continue to run in the lamina spiralis, forming dense plexuses, and finally lose their white substance as they pass through a series of holes in the membrana basilaris, and enter the ductus cochlearis. Here they terminate in the outer and inner hair cells. Those going to the former pass across the tunnel of Corti. The attachment of the nerve fibres is not to the ends, but to the sides of the epithelial cells.

There are tracts of very delicate nerve fibres running among the attached ends of the epithelial cells of Corti's organ, and which follow the spiral course of the cochlea. These are the **spiral fibres.** Their destination is unknown.

The entire perilymphatic space of the internal ear forms a continuous cavity. The semicircular canals and the scala vestibuli open into the vestibule, while the scala tympani is continuous with the scala vestibuli at the apex of the cochlea by an opening called the **helicotrema.** This perilymphatic space is, furthermore, continuous with the subdural cavity of the brain—according to some, by the continuation of this latter cavity along the auditory nerve; according to others, by means of the *aqueductus cochleæ,* which opens into the lower part of the scala tympani. On this account the aqueductus cochleæ has been named the **ductus perilymphaticus.**

The endolymphatic spaces form also a continuous cavity. The membranous semicircular canals open into the utricle; the saccule communicates with the ductus cochleæ by the canalis reuniens. From the utricle a canal proceeds, which unites with a similar canal from the saccule, and the junction of these forms the *aqueductus vestibuli.* This canal is generally supposed to terminate by a blind extremity in the interior of the cranium, under the dura mater; but Hasse maintains that the subarachnoid space of the brain communicates by the aqueductus vestibuli with the endolymphatic cavities of the internal ear. He consequently calls the aqueductus vestibuli the **ductus endolymphaticus.**

The internal ear is altogether unsuited for microscopic preparation by beginners.

The semicircular canals and utricle and saccule are best got from the skate. In this animal the cranium is cartilaginous, and the structures in question can be dissected out with a knife. They are best hardened in osmic acid, or a mixture of chromic acid and spirit. The sections must be very thin, and are best mounted in glycerine.

In order to see the neuro-epithelium, torn preparations may be made from the maculæ or cristæ acusticæ, hardened in osmic acid.

The cochlea is best got from the guinea-pig, since in this animal it projects free into a cavity surrounded by bone, called the tympanic bulla. With a little care it may be separated with sharp bone forceps. It should be hardened either in osmic acid or in a fluid made by adding slowly 180 cc. methylated spirit to 20 cc. of a five per cent. solution of chromic acid. This fluid should be changed every two or three days for ten days. The cochlea is then placed in chromic and nitric acid, which should be frequently changed until the bone is softened. Having been well washed in water, it is soaked with gum, and then placed in spirit, which hardens the gum in the interstices of the organ, and gives support to the fragile structures while the sections are being made. The sections should be parallel to the axis of the cochlea, and it is only two or three sections near the axis that are of use. The sections must be placed in water, to dissolve out the gum, then stained in carmine or logwood, and mounted in glycerine (Rutherford).

Instead of gum, paraffin may be used as the embedding mass. The paraffin method will be described in the Appendix.

CHAPTER XIX.

THE MUCOUS MEMBRANE OF THE NOSE.

THE lower portions of the nasal passages belong to the **respiratory tract,** and, like the remainder of this, are covered by a columnar ciliated epithelium, with goblet cells. Numerous acinose glands, some of a mucous, but the greater number of a serous type, open on the surface. The blood-vessels of the mucous membrane are numerous and large, particularly the veins, which lie very superficially. Bundles of smooth muscular tissue traverse the mucous membrane between the glands and large blood-vessels. This arrangement of dilated blood-spaces surrounded by muscular bands has some resemblance to a cavernous tissue (Klein).

The proper **olfactory region** of each nasal cavity is confined to its upper part, the superior and part of the middle turbinated bones, the superior meatus, and the corresponding parts of the septum. In these places the mucous membrane is characterised by having a deep yellowish-brown colour.

The **epithelium** of this region is peculiar in its structure, and contains elements which are, in all probability, the terminal organs of the olfactory nerves. It is consequently an example of neuro- or nerve-epithelium. It is much thicker than the epithelium of the respiratory region, and consists of **three kinds of cells,** all of which are elongated bodies, placed at right angles to the basement membrane on which they rest.

1. Long **columnar-shaped cells,** each of which

contains an oval nucleus, situated somewhat above (that is, nearer to the surface than) its middle part. The part of the cell above the nucleus is cylindrical and longitudinally striated; while below the nucleus, the cell is diminished in thickness, and marked by numerous rounded depressions, which are separated by crests or ridges. At its deep or attached end the cell expands into a conical foot, or divides into short processes, which are attached to the basement membrane. The protoplasm of these cells sometimes undergoes a mucous transformation, or, in other words, the cells become goblets. This change is, however, not so frequent in the olfactory portion of the nose as it is in the respiratory region.

The pigment which gives the mucous membrane its brown colour is in great part contained in the epithelial cells of the first kind. In men it is situated in the superficial part of the cells, while in animals it occupies the portion next the basement membrane.

2. Each of the **cells of the second kind** possesses a round or short oval nucleus. About this is only a small quantity of protoplasm, massed chiefly at the superficial and deep poles of the nucleus. From the former a moderately thick cylindrical process extends to the free surface of the epithelium, where it terminates, in men and mammalian animals, in a short, rod-like end; but in many of the lower animals it carries on its free extremity a bunch of long, hair-like appendages, somewhat resembling cilia, from which, however, they differ in having no power of movement. From the protoplasm at the deep pole of the nucleus a delicate varicose thread is continued downwards, and is supposed to become continuous with the terminal fibres of the olfactory nerve. These cells are, therefore, looked on as the **end organs of the nerve of smell,** while the cells of the first kind are merely supporting organs. The rounded depressions which were noticed as occurring on the deeper part of the supporting cells

are caused by the pressure of the nuclei of the olfactory cells. These nuclei lie in several layers, so that some cells have a short external and a long internal process, and others just the reverse, according as the nucleus which divides the two portions of the cell lies in a superficial or in a deeper layer. The nuclei of the supporting cells all lie at the same level, and on a plane superficial to the nuclei of the olfactory cells.

3. The **cells of the third kind** are usually described as short conical bodies, whose broad ends rest on the basement membrane, and whose apices extend among the deeper parts of the other cells. They contain oval nuclei. By most histologists they are looked on as **replacement cells,** or young forms of the first and second kind. Ranvier, however, describes them as being flat, of stellate shape, spread out on the surface of the basement membrane, and anastomosing by their processes. He thinks that they have no relation to the other cells, and he looks on them as being analogous to the flat cells which occur in the external reticular layer of the retina. He calls them **basal cells.**

A fine cuticulum, called the **membrana limitans olfactoria,** exists on the free surface of the epithelium of the olfactory region. It covers over the ends of the supporting cells, but is perforated by holes, through which protrude the rods or hairs of the olfactory cells.

The **mucous membrane** is thicker than in the respiratory region, and contains the bundles of the olfactory nerve, and peculiar glands, called after their discoverer, **Bowman's glands.** They are tubular, slightly branched, and lined by polygonal granular epithelial cells. They open on the surface by a narrow duct, which traverses the thick olfactory epithelium, and consists of a basement membrane, lined by small, flat cells. In man, acinose mucous glands, or transitional forms between these and Bowman's glands, are commonly found.

The fibres of the **olfactory nerve** consist each of an axis cylinder, composed of very fine fibrils, surrounded by a neurilemma, with numerous nuclei. They have no white substance of Schwann, resembling in this the fibres of the sympathetic nerve, from which, however, they differ in being thicker, and in not forming anastomoses with one another. They run in bundles, each of which is surrounded by a connective-tissue sheath, somewhat resembling the perineurium of other nerves. Between the laminæ of this sheath are continous lymphatic spaces. The nerve bundles divide, and form plexuses at different depths in the mucous membrane, and in the most superficial plexus the nerve-fibres divide into fibrils or groups of such. These penetrate the epithelium, and are believed to become continuous with the fine varicose threads which form the deeper part of the olfactory cells, and which they very much resemble in their appearance. According to Ranvier, the continuity of the nerve-fibrils with the cells is not direct, but the fibrils form a plexus, which lies superficial to the basal cells of the epithelium, and with the threads of this plexus the deep processes of the olfactory cells unite.

The **lymphatic spaces** about the nerves, as well as the general lymphatics of the olfactory mucous membrane, can be injected from the subdural space of the brain (Key and Retzius).

Lying at each side of the septum of the nose, at its anterior and lower part, is the **organ of Jacobson.** This is a short tube, closed posteriorly, and opening in front either into the nasal cavity or into the canal of Stenson. It is compressed from side to side, and on cross section has a somewhat reniform shape. Its inner wall is covered by epithelium, resembling that which lines the olfactory region; while its outer wall is covered by ordinary columnar ciliated cells, with goblets. Numerous acinose glands pour their secretion into the tube, chiefly at the upper and lower angles. The inner wall receives a branch of the olfactory nerve. In the outer wall numerous large blood-vessels are contained. The entire organ is supported, in most animals, by a special piece of cartilage ; but in the mouse it is enclosed in a thin, bony capsule. Between the capsules of the two sides the lower part of the cartilaginous septum of the nose is received. In this animal the organ of Jacobson undergoes a kind of rotation posteriorly, or towards its closed end, so that the side lined by sensory epithelium, instead of lying internally, is inferior, and the side lined by ordinary columnar cells is superior, the whole tube being compressed from above downwards.

1. In a frog the nasal canal is very short and simple. The posterior nares open just within the margin of the upper jaw. A frog is decapitated, and the nasal canals slit up with pointed scissors. The head is placed to macerate in diluted Müller's fluid or dilute alcohol for twenty-four hours. If the inside of the nose be then scraped, in the scrapings will be found, besides ordinary ciliated and goblet cells, the two first kinds of cells peculiar to the olfactory region. They may be stained in picrocarmine, and mounted in glycerine.

The elements are very fragile, and often become twisted and broken in the process of mounting. In order to give them more resistance, Ranvier recommends that the fresh mucous membrane should be placed for an hour in dilute alcohol, then for five minutes in osmic acid, one per cent., and then in water. The cells are then scraped off the membrane, and separated in water or picrocarmine. In the frog the free ends of the olfactory cells have long, hair-like processes, which are very delicate, and are frequently destroyed in making the preparations.

2. The superior turbinated bone or the upper part of the nasal septum of a recently-killed rabbit is placed in chromic acid and spirit for a week ; then in chromic acid and nitric acid until the thin bone is softened ; then washed in water, and placed in alcohol. Thin vertical sections are stained in logwood, and mounted in balsam or glycerine.

The distinction between the non-olfactory parts of the mucous membrane, with its ciliated epithelium, and the olfactory portion, with its much thicker sensory epithelium, will be at once apparent. In the latter there will be seen a continuous row of oval nuclei near the surface. These belong to the supporting cells. Below them are several layers of round nuclei, somewhat resembling the granule layers of the retina. These belong to the olfactory cells. At the deeper parts may be seen the nuclei of the basal cells. In the mucous membrane Bowman's glands are conspicuous objects, and some of their ducts will probably be met passing through the epithelium. The bundles of the olfactory nerve will also be seen.

In the deeper layers of the epithelium and in Bowman's glands pigment is found.

3. The head of a recently-decapitated mouse is placed for a few days in chromic acid and spirit, and then in chromic and nitric acids until decalcified; then washed, and preserved in spirit. Transverse sections are made through the nose, perpendicularly to the plane of the palate. Stained in logwood and eosine; mounted in dammar.

A general view of the chambers of the nose will be got, and those parts which are lined by ordinary epithelium may be distinguished from those which bear sensory epithelium. The large veins will probably still contain some blood, especially if the animal has been killed by chloroform, and the neck ligatured above the line of decapitation. The blood will be coloured red by the eosine. At each side of the lower part of the nasal septum will be seen the organ of Jacobson, appearing on cross section as an oval opening, compressed from side to side. On its inner side the epithelium is similar to that on the olfactory mucous membrane, but its outer side has ordinary ciliated cells. Some acinose glands open into the tube, specially at its lower angle. The incisor teeth are very beautiful objects. In rodent animals these teeth, like hairs, are always growing. On their upper aspect will be seen the regular columnar cells, which are destined to form the enamel. On the lower surface this is absent, and the dentine will be seen firmly attached to the bone by strong fibrous bands.

Such a section is most instructive, in consequence of the numerous objects included in it, as muscle, glands, nerves, vessels, epithelium of various kinds, bone, cartilage, teeth, hairs, &c. It furnishes a valuable exercise in microscopic diagnosis.

CHAPTER XX.

THE ORGANS OF TASTE.

The organs of taste consist of little oval masses of modified epithelial cells. From their shape and the arrangement of the cells composing them they are called **taste-buds.**

They occur on the sides of the circumvallate papillæ, and on the outer side of the groove surrounding them, on the fungiform papillæ, and on the laminated organ called the papilla foliata, which is only rudimentary in man, but highly developed in the rabbit. They are found also on the soft palate and epiglottis, and even deeper in the larynx.

Each taste-bud is an oval body whose long axis is placed vertically or obliquely to the surface: one small end reaches the free surface, the other rests on the basement membrane, while the sides of the organ are surrounded by the ordinary cells of the compound scaly epithelium.

In each taste-bud **two kinds of cells** are found —one flattened, spindle-shaped, frequently branched at its lower part, and containing a round or oval nucleus. The other possesses an oval nucleus surrounded by a small quantity of protoplasm : from this the middle part of the cell, two processes are given off. One extends towards the surface, and terminates n a thin rod-like extremity, which projets from the free end of the taste-bud. The other is much thinner, and passes to the deep end of the organ, where it sometimes divides and is believed to become coninuous with a nerve fibril. These latter cells are the sensory epithelium. The others are supporting cells. Among the former certain varieties have been described (Schwalbe. Krause). The two kinds of cells are generally supposed to be so arranged that the supporting cells form a sheath, something like the calyx of certain flowers, in the interior of which the sensory cells are contained, like the stamens and pistil. The outer end of the calyx opens on the surface by a narrow pore, through which project for a short distance the rod-like extremities of the sensory cells. Ranvier has, however, shown that in the interior of the taste-bud sensory and supporting cells lie side by side, forming an arrangement much more like that met with in the olfactory and other sensory epithelia.

Nerves, branches of the glossopharyngeal, can be traced to the taste-buds; and it has been stated by some observers that the fibrils become continuous with the sensory cells. Atrophy of the taste buds has been observed to follow sections of the glossopharyngeal nerve. Large **veins** exist in the interior of the papillæ supplied with taste organs. It has been supposed that a species of erection of these papillæ occurs and favours the acuteness of the perception of sapid impressions.

Underneath these papillæ are situated **serous glands,** whose watery secretion is supposed to wash away matters from the epithelium, and so to prevent the too long continuance of any sensation.

1. The papilla foliata on the rabbit's tongue is the best place to see the taste-buds. This papilla forms an oval patch on each side of the posterior part of the tongue. It is not raised above the surface, but its limits are very clearly defined by its structure; its surface being marked by fine parallel ridges.

The papilla is carefully removed from the tongue of a recently killed animal and placed in diluted Müller's fluid, or dilute alcohol for two or three days. Scraping from the surface may then be isolated in water, stained in picrocarmine, and mounted in glycerine.

The cells of the different layers of the ordinary scaly epithelium will be seen. Some of the superficial scaly cells will be met, which have a deep notch in one edge, or are perforated by a round hole. These are cells which bordered the superficial end of a taste-bud. The flattened spindle-shaped supporting cells will be readily seen. The delicate sensory cells are more difficult to get perfect.

2. The fresh papilla foliata is hardened in chromic acid and spirit for a week; then washed and placed in alcohol.

Sections should be made perpendicularly to the surface and at right angles to the ridges; logwood; balsam. Each ridge, seen in section, presents three elevations of the mucous membrane embedded in the epithelium. The central one is highest, and contains in its interior a large vein. On the outer sides of the two lateral elevations are seated the taste-buds. They will be seen, lying from above downwards, in series of from two to four on the sides of the ridges.

Under the papilla will be seen the serous glands which lie among the muscular fibres and whose ducts open between the ridges.

CHAPTER XXI.

THE NERVE CENTRES.

THE nerve centres, consisting of the brain and spinal cord, are covered by certain sheaths, or membranes, formed of connective tissue. These are three in number.

The most external is called the **dura mater.** It is composed of firm fibrous tissue, the bundles of which cross each other in the cerebral part, but in the spinal canal run mostly parallel to one another and to the cord. Between the bundles is a system of juice canals, which contain the flat connective-tissue cells, and which open by stomata on the outer and inner surfaces of the membrane. In the spinal canal the outer surface of the dura mater is free, and is covered by a continuous layer of endothelium. In the cranium there is a close attachment between the bone and the dura mater at the base, and at the convexity along the sutures. Elsewhere an interval exists between the bone and the outer surface of the dura mater, and corresponding to this interval, the dura mater is covered externally by endothelium. The space superficial to the dura mater is called the **epidural space.**

The dura mater is supplied by blood-vessels from the meningeal arteries. In the cranium the capillaries of the inner part of the membrane are in parts dilated into ampullary spaces.

Nerve twigs from the fifth and hypoglossal, as from the sympathetic, go to the dura mater. They supply partly the walls of the vessels, but are in part distributed to the membrane itself (Alexander).

The inner surface of the dura mater is smooth, and is covered everywhere by endothelium. It forms the outer boundary of a space which lies between it and the second membrane, or arachnoid. This space is called the **subdural space.** It is of a lymphatic nature, and communicates in its cranial part with the lymphatic vessels of the neck, and in the spinal portion with those of the back and loins. It also communicates with the lymphatic spaces contained between the perineural lamellæ of the peripheral nerves and the lymph spaces which surround the olfactory, optic, and auditory nerves. Usually the subdural space contains only enough fluid to moisten its surfaces.

The second membranous sheath of the nerve centres is called the **arachnoid**. It is an exceedingly delicate membrane, formed of fine bundles of connective tissue, which cross each other, leaving small interspaces. These, however, are closed by the endothelium, which covers both the outer and inner surfaces of the arachnoid. In the spinal canal the arachnoid lies close to the inner surface of the dura mater, separated from it by only a narrow subdural space, while there is a considerable interval between the inner surface of the arachnoid and the pia mater of the cord. This interval is called the **subarachnoid space,** and is traversed by the ligamentum denticulatum, and, chiefly in the posterior part, by bands and imperfect septa, formed of connective-tissue bundles, clothed by endothelium.

In the cranium the arachnoid lies close to the convexities of the convolutions, separated here by only a very small quantity of tissue from the pia mater; but while the pia mater dips into all the sulci, and is closely in contact everywhere with the surface of the brain, the arachnoid stretches across from one convolution to another, and, corresponding to the depressions between them, is separated by a considerable interval from the pia mater. This interval, or subarachnoid space, is occupied by a loose spongy tissue, formed of connective-tissue bundles, clothed by endothelium, and continuous on the one hand with the deep surface of the arachnoid, and on the other with the superficial aspect of the pia mater. This tissue is called the **subarachnoid tissue.** In places, as around the medulla oblongata, at the base of the brain, and above the corpus callosum, the subarachnoid space is much dilated, and forms enormous sinuses, which all communicate freely with one another, and with all the other parts of the cerebral and spinal subarachnoid space, which thus forms but one continuous cavity.

This cavity communicates also with the intraventricular space of the brain by three openings in the region of the fourth ventricle. One of these openings is situated at the posterior angle of the fourth ventricle, and is called the foramen of Magendie. The others are found, one at each lateral angle of the fourth ventricle. The subarachnoid space, in common with the ventricles, contains a large quantity of fluid, known as the **cerebro-spinal fluid**.

There is no communication between the subdural and subarachnoid spaces, but the latter is continuous with the lymphatic spaces around the spinal nerves, with the lymphatics of the nasal mucous membrane, with the subarachnoid space around the optic nerve, and with the perilymphatic spaces of the internal ear.

There can consequently be no doubt that the subarachnoid, like the subdural space, is a portion of the lymphatic system.

On the outer surface of the arachnoid there are found in many places fungiform projections, which are known as **Pacchionian granulations.** They occur always in the neighbourhood of the venous sinuses, or large veins of the dura mater. They are most numerous along the sides of the superior longitudinal sinus, and are met with also along the straight, lateral, superior petrosal, and cavernous sinuses. Each granulation consists of a spongy tissue, continuous with, and similar in structure to, the subarachnoid tissue, covered by the arachnoid membrane itself. At first sight it appears to project naked into the cavity of the sinus, or vein with which it is in connexion; but more careful examination shows that it is separated from the blood by a greatly thinned extension of the dura mater, and by a narrow interval, continuous, around the generally-constricted base of the granulation, with the general subdural space of the brain. Hence there are three layers of endothelium separating the blood from the interior of the Pacchionian granulation—1. that of the venous sinus, or blood-vessel; 2. that covering the inner surface of the dura mater; and 3. that of the arachnoid itself, continued over the surface of the granulation (Waldeyer).

It has been found that an injection thrown into the subarachnoid space passes freely into the interior of the granulation and into its subdural space, but does not pass back, so as to fill the general subdural cavity. While an injection forced into this latter cavity flows freely into the subdural space of the Pacchionian granulation, but does not extend to the tissue of the granulation itself, or into the subarachnoid cavity. In both cases, however, the injection, whether forced into the subdural or subarachnoid space, passes into the venous sinuses through the granulation. Although, therefore, no anatomical pores are to be demonstrated in the membrane covering the granulations, it appears that fluids flow freely from the latter into the blood-vessels. It was found that the pressure of the subarachnoid fluid in the dog was slightly greater than that of the blood in the longitudinal sinus; hence it is probable that in the Pacchionian granulations a sort of valvular arrangement exists, by which the lymphatic subarachnoid fluid can flow off by the blood-vessels (Key and Retzius).

Waldeyer has found also, that injections forced into the subdural space pass into lacunæ in the dura mater unconnected with the Pacchionian granulations, and thence into the blood. These lacunæ are believed by some to be of a lymphatic nature, but are more probably widened blood capillaries.

The third membrane, or **pia mater,** lies everywhere in close contact with the surface of the brain and cord, and sends processes into these parts. On the spinal cord the pia mater consists of two layers. The outer layer is formed of longitudinally-arranged bundles of connective tissue, covered by endothelial cells, and is continuous by its outer surface with the trabeculæ of the subarachnoid tissue. The inner layer, or intima piæ, is formed of bundles of fibrous tissue, running for the most part circularly, and crossing each other at acute angles. External to this on both surfaces is a network of elastic tissue, and the whole is covered both externally and internally with endothelial cells. Pigment cells are sometimes found in the intima piæ, most commonly in the upper part of the cord.

The blood-vessels run between the external and internal layers of the pia mater, and funnel-shaped extensions of the intima piæ are continued along the fine vessels which penetrate the spinal cord, forming for them a loose adventitious coat, the spaces in which can be injected from the subarachnoid cavity. These are the so-called perivascular lymphatic spaces of the vessels of the cord.

A fissure of a lymphatic nature exists between the two layers of the spinal pia mater.

The pia mater of the brain consists of only one layer, which corresponds in structure to the intima piæ of the cord. On the outer surface of this membrane the blood-vessels ramify in enormous numbers, and it is only when they have become reduced to very small twigs, with the structure of capillaries, that they penetrate the substance of the convolutions. Here, as in the cord, they are accompanied into the nervous mass by extensions of the pia mater, whose meshes can be injected from the subarachnoid space.

A space between the pia mater and the surface of the brain and cord has been described under the name **epicerebral,** and **epispinal space.** Such a space has probably no real existence. The spaces between the adventitious coats of the blood-vessels and the substance of the brain and cord, as well as the supposed lymphatic spaces surrounding the ganglionic cells of these parts, are also artificial, and owe their origin to shrinking of the tissue during the process of hardening.

The pia mater sends processes into the interior of the brain, between the inferior surface of the cerebellum and the upper surface of the medulla oblongata, and through the great transverse fissure of Bichat. Each of these processes consists of two layers of pia mater, with interposed subarachnoid tissue.

Connected with these are structures known as **choroid plexuses.** They are fringe-like masses, formed of branching processes of connective tissue, containing a very abundant plexus of capillary vessels. They are covered by a layer of flat epithelial cells, which are part of the lining of the ventricles of the brain. The choroid plexuses are therefore not contained in the interior of the ventricular space.

The pia mater is abundantly supplied with nerves, not only from the sympathetic, for the supply of the numerous blood-vessels, but also with cerebro-spinal fibres; in the cord with branches from the posterior nerve roots, and in the brain from the 3rd, 4th, 5th, 10th, and 11th cranial nerves.

Nerves have not certainly been demonstrated in the choroid plexuses.

The nerve centres are composed of nerve fibres, ganglionic cells, and a peculiar supporting or connective tissue called neuroglia.

The neuroglia is a tissue whose true structure is as yet but imperfectly understood. It contains very fine fibrils, which form an extremely delicate network, and which are commonly supposed to be closely allied to the fibres of elastic tissue (Gerlach).

There are also, in some places much more numerous than in others, peculiar cells with a small body, but with long branching processes. These cells vary greatly in shape, and are called from their discoverer the cells of Deiters. Ranvier has recently studied the neuroglia cells, and finds that in their most developed form they consist each of a flattened protoplasmic mass, which contains the nucleus. From this come off long branching threads, which, however, are not mere extensions of the protoplasm, but consist of a substance different from this. They can be traced through the mass of protoplasm in which they are merely embedded, but with whose substance they are not continuous. Besides these there are round or polyhedral cells, and branched cells whose processes are protoplasmic. According to Schwalbe, many of the cells of the neuroglia are merely wandering cells, such as are found in most of the tissues of the body.

Between the cells and fibres is a substance which, in preparations prepared by chromic acid or its salts, or by alcohol, appears finely granular, but which in the fresh condition is structureless. This is compared by Schwalbe to the cementing substance which holds together the cells of epithelium, but its real nature must be admitted to be still doubtful.

In parts, a very finely reticular substance occurs, such as was described in the reticular layers of the retina, and which, like this, resists artificial digestion. It is hence believed to be of a horny composition, and is called the hornspongiosa of the central organs (Kühne. Schwalbe).

The neuroglia, composed of these elements, differs greatly in quantity and in consistence in different parts of the nerve centres.

I.—*The Spinal Cord.*

The spinal cord varies greatly in thickness in different parts. It is largest in the cervical swelling, thinnest in the dorsal region, and of medium thickness in the lumbar enlargement. Below this it thins to a point: this conical part is called the conus medullaris; it terminates in a thread, the filum terminale. All along the anterior surface of the spinal cord in the middle line a deep groove is seen. This is the anterior median fissure, and contains processes from the pia mater and blood-vessels. Opposite to this, along the posterior surface, is the posterior median fissure, which is not a true fissure, but merely one of the extensions from the intima piæ, already noticed. By the two fissures the cord is divided into lateral halves. Each of these is further divided by the exit of the nerve roots into an anterior, lateral, and posterior column: the first extending from the anterior median fissure to the line of anterior nerve roots; the second from this to the line of posterior nerve roots, and the third from the posterior roots to the posterior median fissure. Since the anterior nerve roots do not come off in a continuous line, but leave intervals between successive roots, in places there is no visible division between the anterior and lateral columns, and they are sometimes spoken of together as the antero-lateral column. Finally, in the cervical region a process of pia mater, extending into the cord at each side of the posterior median fissure, subdivides the posterior column into an internal division, the *funiculus gracilis* or column of Goll, and an external, the *funiculus cuneatus*, or column of Burdach.

On a transverse section through the spinal cord we readily recognise the **anterior** and **posterior median fissures,** dividing the section into two symmetrical lateral portions, which are connected across the middle line by a narrow bridge. This bridge consists of the commissures. The anterior portion, which forms the floor of the anterior median fissure, is composed of nerve fibres, and is called the **anterior** or **white commissure.** The posterior portion, which forms more than half the entire depth of the commissure, is composed of grey matter, forms the floor of the posterior median fissure, and is called the the **posterior** or **grey commissure.** About the middle of the grey commissure is seen an opening, whose shape varies in different parts of the spinal cord, and which is the cross section of a canal **(the central canal)**, which runs the whole length of the cord, opens above into the fourth ventricle at its inferior angle, and below, after having passed through a dilatation in the lower portion of the conus, and which is called the **ventriculus terminalis,** is continued into the filum terminale where it ends blindly. In young animals the canal is lined by columnar ciliated epithelium, but in the adult man it is frequently obliterated, either in its entire length or in places, by epithelial debris, or by a connective-tissue growth, its position being marked only by a group of nuclei. It is surrounded by a portion of so-called **gelatinous substance,** that is, a portion of grey matter mainly composed of neuroglia. When the epithelium persists, the attached ends of the cells are continued as fine threads among the fibres of the surrounding neuroglia. The gelatinous substance surrounding the central canal is spoken of as its **ependyma.** It is continuous with a similar layer of connective tissue which forms the boundary of the vertricles of the brain.

The proper nervous portion of the grey commissure is separated by the central canal and its ependyma into an anterior and a posterior division. These coalesce at the lateral portions of the commissure. Attached to them are two large masses of grey substance, one on each side. Each mass is of somewhat crescentic shape, with the concavity turned outwards, and occupies the interior of the lateral portion of the cord of its own side. The anterior extremity of each mass is more or less rounded, and never reaches the surface of the cord. The posterior extremity is pointed, and is everywhere connected with the surface either by nerve roots, blood-vessels, or connective tissue. These extremities are called, respectively, the **an-**

terior and posterior horn of grey matter. Since the anterior nerve roots come off from the anterior, and the posterior roots from the posterior horn, these horns divide the section into its three columns on each side; and since the anterior roots run an oblique course from their entrance into the cord to their origin in the grey matter, they are never cut in their whole length by a transverse section. Hence the separation between the anterior and lateral column is in such a section always incomplete.

The size and shape of the **lateral grey masses** vary greatly in different parts of the cord. The amount of grey matter in any part is always directly proportional to the size of the nerve roots given off by this part. Consequently it is greatest in the lumbar, least in the dorsal, and of medium amount in the cervical portion.

From the concave or outer side of each grey mass processes extend into the lateral white column, and, specially in the upper portion of the cord, appear as a network, in the meshes of which lie sections of the nerve fibres of the white substance. These processes are called the **processus reticularis.** In the cervical and upper dorsal regions a triangular-shaped process of grey matter protrudes from the outer and posterior part of the anterior horn into the lateral column, anterior to the processus reticularis. It is called the **tractus intermedio-lateralis,** and is sometimes spoken of as the **lateral horn.**

The grey substance, consisting of the two lateral masses united by the grey commissure, has on cross section the shape of the letter H. It is composed of neuroglia, and a very great number of nerve fibres of extreme minuteness, and which for the most part want the white substance of Schwann, although medullated fibres are much more numerous than was formerly supposed. Its most distinguishing characteristic, however, is the presence of **ganglionic nerve cells**. These cells differ greatly in size, shape, and number in the different portions of the cord. They are largest in the anterior horns, where they have the following structure. The body of the cell contains a large spherical nucleus in the interior of which is a distinct nucleolus. There is also very frequently a mass of yellowish-brown pigment contained in the body of the cell. From the body come off a large number of processes, which extend chiefly in a horizontal direction. These processes are of two kinds: one process of each cell does not branch, but runs forwards, and, being soon clothed with white substance, becomes a perfect nerve fibre, and passes as such into one of the anterior nerve-roots. All the other processes divide and subdivide, until an enormous number of most minute threads result. These form a considerable part of the fibres of the grey matter, but their course and attachments are still only a matter of conjecture. The unbranching process of the cell is called the **axis-cylinder process,** the others the **protoplasmic processes.** Both the processes and the body of the cell itself have a more or less distinctly fibrous structure. The cells in the anterior horns lie in groups, but in most parts of the cord these are badly defined.

The cells in the tractus intermedio-lateralis, and those which lie in the processus reticularis, resemble the cells of the anterior horn. In the posterior horn the cells are for the most part smaller, fewer, and less branched, and do not lie in groups. It is not known that they have two kinds of processes, but it cannot be positively affirmed that they have not. Nothing certain is known of their attachments.

A remarkable tract of grey matter, consisting of nerve fibres and large multipolar cells, is found in the dorsal and upper lumbar regions of the cord. It lies on each side posterior and external to the central canal, in the root of the posterior horn, and separated by only a small part of this from the posterior white column. It presents on transverse section a well-defined rounded figure. It is known as the **posterior vesicular column of Clarke.** The cells are somewhat spindle-shaped, and branch chiefly up and down. These cells would appear to possess axis-cylinder processes which have been traced into a tract of nerve fibres lying on the surface of the posterior part of the lateral column, and called the direct cerebellar tract (Pick).

The apex and sides of the posterior horn are covered by a substance consisting mainly of connective tissue, and which on transverse section has a V shape. This is called the **gelatinous substance of Rolando.** It differs from the connective tissue about the central canal in containing nerve cells.

The surface of the spinal cord is covered by a layer of neuroglia, which sends processes into the white matter.

The **white substance** of the cord is composed of medullated nerve fibres, which, however, have no neurilemma, but are supported merely by the neuroglia which lies between them. It is not known that they have nuclei or Ranvier's constrictions.

The greater number of the fibres run in the length of the cord, and, consequently, on cross section of this appear cut transversely. The axis cylinder appears as a small point surrounded by a clear space, the section of the white substance of Schwann. The fibres vary greatly in diameter: those in the anterior columns are nearly all thick, and those in the posterior columns nearly all very fine; while in the lateral columns thick and thin fibres are mixed.

In **the anterior commissure** the fibres run nearly horizontally, that is in the plane of the section. They pass apparently from the anterior horn of one side to the anterior column of the opposite.

The **anterior nerve roots** are composed of thick fibres. They pass from the anterior horns in bundles downwards, forwards, and outwards, through the white matter to leave the cord. It is certain that some of their fibres are directly connected with the axis-cylinder processes of the nerve cells of the anterior horns. That all their fibres are so connected is not certain, but highly probable.

The **posterior nerve roots** consist of fine fibres. They enter the cord as compact bundles, but soon break up into several smaller bundles, which divide and anastomose. Some of the fibres pass directly into the posterior horns, where many of them, before terminating, turn round and run for some distance upwards, and perhaps downwards in the cord. Others pass into the outer part of the posterior columns, where they ramify, often for a considerable distance upwards or downwards, before they finally turn outwards to enter the side of the posterior horn. Of the further course of the posterior roots in the cord nothing certain is known, at least of an anatomical nature.

In the upper part of the **filum terminale** the continuation of the central canal is surrounded by gelatinous connective tissue, and some nerve fibres running longitudinally. There are also some cells, presumably of a ganglionic nature. About the middle of the filum the central canal ends, and beyond this the filum terminale consists only of pia mater enclosing blood-vessels, connective tissue, and a few nerve fibres.

Microscopic examination of the adult spinal cord has thrown little light on **the course of the fibres** in this organ. Whatever knowledge is possessed on this subject has been gained by physiological experiment, combined with pathological and embryological research. By these means it has been ascertained that the white matter, which appears, when examined by ordinary methods, to be one whole, consists of several distinct tracts, which are developed at different periods, which undergo morbid changes separately, and which subserve very different functions. Some of these tracts vary in size in the different parts of the cord, increasing and diminishing in the same ratio as the grey matter and the nerve roots. Others increase in size steadily from below upwards. These latter are found chiefly in the internal part of the anterior columns bordering the anterior fissure, in the posterior part of the lateral columns, and in Goll's columns. These tracts are so considerable that, taken as a whole, the white matter of the cord increases continuously from below upwards. Of the two classes of white tracts of which we have spoken, those that vary with the grey matter are most probably commissural between different parts of the cord itself, while those which increase from below upwards join portions of the cord with the brain.

That any fibres of the anterior nerve roots pass to the brain directly, that is, without entering into connexion with cells in the spinal cord, is highly improbable. It is true that the depth of the anterior commissure varies directly with the size of the anterior nerve roots; but the fibres in the latter are thicker than those in the commissure, so that a direct passage of one into the other is, to say the least, unlikely. We have good reason for believing that the fibres of the anterior nerve roots all arise from the axis-cylinder processes of the large cells of the anterior horn. The conductors from the brain must therefore enter into connexion with these cells by their protoplasm processes; but this connexion, although a reasonable matter of belief, has not been anatomically established.

As to the posterior nerve roots, we are in a still worse condition of ignorance, for we do not know even their proximate termination in the cord. Since the grey commissure varies in depth with the size of posterior roots, it is possible that a decussation of posterior root fibres takes place, and there is physiological evidence to support such a view; but whether this occurs before or after the root fibres have entered into connexion with nerve cells we know not. We know, however, that while the anterior roots pass directly into the grey matter, a great part of the posterior roots pass upwards and downwards in the outer part of the posterior white columns before entering the posterior horns. These roots form a large part of the posterior columns, and in disease affecting these columns we have evidence of this in the disturbances of sensation which manifest themselves both as anæsthesia and as pain.

The spinal cord is abundantly supplied with blood-vessels. The grey substance is far more vascular than the white.

II.—*Cerebellum.*

It would be impossible, in such a work as this, to describe in detail the complicated structure of the medulla oblongata and basal parts of the brain. A brief description of the grey matter which forms the cortex of the cerebellum and of the cerebrum is all that can be attempted.

The peculiar lamellated structure of the peripheral parts of the cerebellum is too well known to require any notice. On a vertical section the grey substance on the surface can be seen with the naked eye to consist of two layers—an inner, of a reddish colour, the so-called rust-coloured layer; and an outer, of a pure grey colour. Between the two is a fine white line.

1. The **inner layer** is composed mainly of what appear at first sight as naked nuclei, but which are really cells, as each nucleus is covered by a thin layer of protoplasm. These cells lie very closely together, the interspaces being occupied by neuroglia, blood-vessels, and a network of fine nerve fibres. It is most probable that the minute cells, or granules, as they are called, of which this layer is composed are in part small ganglionic cells, and in part belong to the connective tissue. It is, however, not easy to say of any particular cell what its real nature is.

2. Exterior to the rust-coloured layer is a single layer of large multipolar ganglionic cells—the **cells**

of Purkinje. Each cell is flask-shaped, and contains a large nucleus and a nucleolus. From its inner end there comes off a single process, which is unbranched. It passes into the inner layer, and is soon lost among the granules. It is generally considered as the axis-cylinder process of the cell, and is found to assume a coat of white substance, and so to become a nerve fibre. From the outer end of the cell there comes off usually a single process, which soon divides into two, but sometimes two branches come off directly from the cell. These primary branches extend towards the surface into the outer layer, dividing and subdividing until the resulting fibrils are reduced to the most extreme minuteness. The termination of these fibrils is, like that of the fibrils resulting from the branchings of the protoplasm processes of the cells in the cord, very uncertain. They are generally lost from view when they have attained a certain degree of fineness. According to some observers, they become continuous with minute ganglionic cells in the outer layer; and, according to others, they bend back into the inner layer, and become axis cylinders of nerve fibres. The branching of Purkinje's cells takes place only in a single plane—namely, transversely to the course of the white lamellæ of the arbor vitæ; so that the branching is seen only in sections, which are parallel to the course of the white fibres in the central stalk of the lamellæ. The long axis of the cell and its branches generally are directed perpendicularly to the surface. These points must be borne in mind when making sections of the cerebellum.

3. The **outer layer** is called the pure grey layer, or molecular layer, because it is composed mainly of a substance which, in ordinary preparations, presents a finely granular or molecular appearance. In this are contained, besides the branchings of Purkinje's cells, small irregularly-shaped cells, scattered without order. Their real nature is uncertain. Very fine nerve fibres also occur.

Passing in from the surface are connective-tissue fibres, which at their outer ends are attached to the pia mater by an expanded base, somewhat resembling the termination of Müller's fibres in the membrana limitans interna of the retina.

It is probable that the greater part of the molecular layer of the cerebellum is composed of neuroglia. Numerous vessels pass perpendicularly into the grey substance from the surface. The white substance of the cerebellum is composed of fibres, which are finer than those of the spinal cord, and, like them, want a neurilemma.

III.—*Cerebrum.*

The grey substance of the cerebral cortex is penetrated everywhere from its deep surface by bundles of medullated nerve fibres, which pass into it at right angles to the line of junction of the grey and white matters. These bundles can be traced a little more than half way to the surface, where they are lost. It has, however, been recently shown that medullated fibres, in much larger number than was supposed, exist in all layers of the cortical substance, even up to the very surface (S. Exner).

Between the nerve fibres are placed the ganglionic cells embedded in neuroglia. The cells are very numerous, and lie in several superposed layers. Different divisions of these layers have been proposed. That which is generally received, and which corresponds best with what is seen in most convolutions, is the classification of Meynert into five layers. These are, reckoning from the surface—1, a layer containing few or no nerve cells, but consisting of nerve fibres, many of which run parallel to the surface; and of neuroglia; 2, a layer of small, closely-placed, pyramidal cells; 3, a much broader layer of large, less closely-placed pyramidal cells; 4, a layer of small, closely-placed, irregularly-shaped cells; 5, a layer of spindle-shaped cells, whose long axes are parallel to the surface. The first three layers are very distinct; the two inner less so.

The greater number of the cells of the cortex of the cerebrum have a pyramidal shape. They lie so that the apices are directed towards the surface. From the apex of each there comes off a single process, which can often be followed for a great distance, and which sooner or later branches. From the base a central process comes off, which does not branch, and which is supposed to be the axis-cylinder process, and destined to become a medullated nerve fibre. From the peripheral portions of the base several processes arise, all of which branch, and, like the apicial process, form a maze of minute fibrils, which it is impossible to follow to their terminations.

The body of the cell contains a large oval nucleus, and in the larger cells frequently a mass of brown pigment granules. In the deeper parts of the third layer are found groups of extremely large pyramidal cells, known as giant cells. They are most numerous in those parts of the cortex that are believed to have motor functions—namely, the two ascending convolutions and the paracentral lobule.

By certain methods of preparation tracts of nerve fibres can be seen running through the grey matter parallel to the surface. One occurs in the outer part of the third layer, and another near its inner boundary. They are known as the **tracts of Gratiolet.** Although the five layer type is the most general, in some convolutions other arrangements exist. In the occipital lobe Meynert describes eight layers, for the most part alternations of layers of pyramidal and irregular-shaped cells. In the hippocampus major large pyramidal cells predominate; and, in the claustrum, spindle-shaped cells.

Besides the nerve cells and fibres, much connective tissue (neuroglia) exists in the grey matter, and its numerous nuclei must not be confounded with those of the ganglionic cells. The latter are, as a rule, larger, and stain less deeply.

The blood-vessels pass in perpendicularly from the pia mater, and, branching at right angles, form a very abundant capillary plexus in the grey matter. They are surrounded, near their entrance, by funnel-shaped prolongations from the pia mater. These are the perivascular lymphatic sheaths; they communicate freely with the subarachnoid cavity. There are no proper lymphatic vessels in the brain or spinal cord.

A fresh **spinal cord,** human, dog, cow, sheep, is carefully removed, and cut into pieces not more than one inch in length. It is well not to completely separate the pieces, but to leave them attached by the pia mater on one side, so that the relative position and the portion of the cord to which each piece belongs may afterwards be known. The whole cord is suspended in a large quantity of two per cent. bichromate of ammonia, which should be changed on the second day, and again before the end of the week. After a month, remove the cord from the bichromate, and wash in water until all yellow colour is removed; then place in alcohol, which must be changed two or three times at first.

Instead of bichromate of ammonia, Müller's fluid may be used to harden the cord. It requires four to eight weeks. The fluid should be used in large quantity and frequently changed. When sufficiently hardened, the cord should be washed, and preserved in spirit until required for section. The process of hardening may be much shortened if it be carried out at the temperature of 30°–40° C. Müller's fluid will harden sufficiently in eight to ten days at this temperature (Weigert). A still more rapid hardening may be effected by the solution employed some years ago by Erlicki, and lately again recommended by Weigert. It consists of bichromate of potass. $2\frac{1}{2}$; sulphate of copper, $\frac{1}{2}$; water, 100. It hardens, at ordinary temperatures, in from eight to ten days; at 30°–40° C., in four days.

1. **Transverse section,** best made with a microtome. Stained in a solution of neutral carminate of ammonia or in cochineal. The stained sections are mounted in balsam.

It is unnecessary to recapitulate the objects which a transverse section of the cord presents for observation. They will be readily recognised from the description already given.

Transverse sections of different parts of the human cord should be examined, in order to see the differences in the amount of white and grey matter, and the varying shape of the latter in the different regions. Also **longitudinal sections,** of which the following are most instructive:—

a. A frontal section, *i.e.* in a plane from side to side, passing through the anterior or white commissure. In the middle the decussating fibres of the commissure will be seen. External to these is on each side the posterior part of the anterior horn of grey matter. Outside all the lateral white columns.

b. A section running in the plane of the passage of an anterior root through the anterior column. The oblique course of the root fibres and the anterior horns will be seen.

c. One through the outer part of the posterior column, to show the passage up and down of the posterior root fibres before they pass into the grey matter.

2. To isolate the nerve cells, the best method is that of Gerlach. The spinal cord of a cow is to be preferred, in consequence of the large size of the cells of the anterior horn. This is so great that, when stained, they can be seen with the naked eye as red points. From the fresh cord as thin sections as possible are made with a razor, and placed in a solution of bichromate of ammonia 1 to 5,000–10,000 water. They are left in this for two or three days, then washed, and placed for twenty-four hours in dilute carmine solution, and then washed again. With the point of a knife small pieces are dug out of the anterior horn, and carefully teased on a slide, on which there is barely enough fluid to keep the preparation moist. The teasing should be done under a dissecting microscope, or after every few touches of the needle the object should be examined with a low power. It is not very difficult to get well-isolated cells; but the axis-cylinder processes are apt to break off close to the cells, at which place they are very thin. In putting on the cover-glass, on which there should be a small drop of glycerine, all the processes are apt to get twisted around the body of the cell, and the appearances of the object are thus destroyed. This is best avoided by covering the object only after it has been allowed to dry on the slide to such a degree that it adheres slightly to the glass.

Maceration of small pieces of the cord for ten to fourteen days in one-tenth per cent. solution of osmic acid, and then for some time in dilute glycerine, allows the cells to be well isolated. It is not necessary to stain them. They are to be mounted in glycerine.

3. A good method for isolating the cells of the neuroglia, and which serves also for the nerve cells, has been recently described by Ranvier. Pieces of the fresh cord, or brain, are placed for twenty-four hours in one-third alcohol (rectified spirit, 1; water, 2). Fragments are then removed from the macerated organ, and shaken in a test-tube with distilled water. By this the cells are separated. Some picrocarmine is added to colour them. They are next allowed to settle to the bottom of the tube, from which they are removed with a pipette, and transferred to another test-tube containing very dilute osmic acid

solution. As they settle through this fluid they become fixed and hardened, so that their shape and structure are well preserved. When they have deposited they are again taken up with the pipette, placed on the slide, and mounted in glycerine.

The cerebellum and cerebrum are to be hardened in bichromate of ammonia, as was directed for the cord. Müller's fluid, or Erlicki's solution, gives good results.

4. Vertical section of the cortex of the **cerebellum** parallel to the white stalks of the leaves of the arbor vitæ; carmine; balsam. A very good stain for the cerebellum and other nerve centres is aniline blue-black (Sankey); it is best used in alcoholic solution, made according to the following formula:—aniline blue-black, 1 decigram; water, 4 cc.; dissolve: add rectified spirit 100 cc. This solution must while acting be kept covered, to prevent evaporation. It dehydrates at the same time as it stains, so that, before placing the sections in oil of cloves, they need only be washed in spirit. If the section is over-stained, the excess of colour is readily removed by alcohol. Prolonged action of spirit removes all the colour.

A mixture of logwood and aniline blue-black gives good results. Logwood stains the granules of the inner layer intensely, but has only slight effect on Purkinje's cells: these are stained by the aniline.

5. Isolation of Purkinje's cells may be effected by the same methods as those employed for isolating the cells of the cord.

6. Vertical section through the cortex of the **cerebrum.** The human brain is best, and the upper portion of the ascending convolutions are to be preferred. Carmine or aniline blue-black; balsam.

The layer on the surface without ganglia. The layer of small pyramidal cells and the broad layer of large pyramids are distinct. The two inner layers less so. Groups of giant cells occur in the third layer. The blood-vessels will be seen passing vertically in from the surface, surrounded by their perivascular sheaths of pia mater.

7. Isolation of nerve cells by the same methods as those given under 2 and 5.

In ordinary balsam preparations the course of the fibres in the grey matter is indistinct, and there can be no doubt that a great deal of what appears as 'finely granular' matter consists really of fibres.

8. The following method, when successful, enables the fibres and cell processes to be admirably seen. A thin section of the hardened brain, or cord, is placed in alcohol for at least ten minutes; the excess of alcohol is removed with filter-paper; the section is then placed in xylol until it begins to become transparent, when it is rapidly covered in balsam (Merkel). The

axis cylinders appear as dark and very distinct lines in the section. Preparations of the cortex of the cerebrum made by this method are of great beauty. Unfortunately they do not keep. After some time they become transparent throughout. Xylol of commerce is not an uniform substance. A certain relation must exist between the strength of the alcohol and the xylol used. This can be arrived at for each specimen of the latter only by trial. If the sections become too transparent the alcohol is too strong; if not transparent enough, it is too weak.

Of the other methods for showing the large number of medullated fibres in the grey matter of the nerve centres, the two following are the best :—

9. Small pieces, not larger than a cubic centimetre, of the fresh organ are placed in at least ten times their volume of one per cent. osmic acid solution. This is changed after two days, and again a few days later. After five to ten days the pieces are removed, washed for a short time in water, placed for a few seconds in alcohol, and then embedded in the usual way in the wax and oil mass. Very thin sections are made with a microtome, and immediately placed in glycerine. From this they are transferred to the slide, on which a drop of solution of caustic ammonia has been placed. This softens the horny matter (neurokeratin) of the neuroglia, and makes it quite transparent. It makes the section very soft, so that great care is required in applying the cover-glass, which should be supported at the edges by bits of paper.

In sections of the grey matter of the cerebral cortex treated in this way the ganglionic cells are scarcely visible, but a surprising number of medullated nerve fibres are seen in all the layers, and, in consequence of their blackness, they present themselves with the most admirable distinctness (Exner).

In this method the most important step is the application of the ammonia. The strong liquor ammoniæ of the Pharmacopœia is too strong; it almost instantaneously reduces the section to a semi-fluid consistence. The exact degree to which the ammonia should be diluted cannot be foretold in each particular case, but it can readily be determined by a few trials. These preparations are difficult to preserve permanently. They very soon spoil.

10. Portions of the nerve centres are hardened in Müller's fluid, or Erlicki's solution. After removal from the chromate solutions they must not lie too long in alcohol. So long as they retain a brown colour they may be used, but once they have become green they cannot be prepared by this method.

Thin sections are cut with a microtome, and placed for some hours in a saturated watery solution of acid fuchsin.* Removed from this, they are washed in water, and transferred to a solution of caustic potash in alcohol. This solution is prepared as follows:—To 100 cc. absolute alcohol one gram fused caustic potash is added, and left twenty-four hours, until the alcohol has dissolved as much as it will. Of this strong alkaline solution 10 cc. are added to 100 cc. alcohol. In this more dilute fluid the sections are placed and moved about until the grey substance appears distinctly lighter in colour than the white. Beyond this point they must not be left in the alkali. They are then placed in water, to remove the potash, and then transferred to fresh water. If now the grey substance is seen to be evidently lighter than the white, the sections are dehydrated in alcohol, cleared in oil of cloves or xylol, and mounted in Canada balsam or dammar varnish. If, however, the difference of colour between the grey and white matter is not sufficiently marked, the sections must be put back into the alkaline potash solution for a short time longer, then washed, and mounted as directed above (Weigert).

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* This, as well as other aniline dyes, can be had retail and of good quality from Messrs. Becker & Co., 34, Maiden-lane, Covent-garden, London.

The white substance of the medullated nerve fibres is stained red. In the grey matter a great abundance of such fibres is seen, and their distribution and course are extremely evident. The ganglionic cells are generally of a bluish colour, but they are made much more distinct if the section, before dehydration, is stained in logwood.

With a little care this method is not difficult to carry out. It gives remarkably clear and instructive preparations.

CHAPTER XXII.

DUCTLESS GLANDS.

There are a number of organs of whose structure little is known, and of whose functions we are entirely ignorant. These organs are called ductless glands. It is supposed by Klein that they secrete something which is carried away by the blood-vessels or lymphatics. The ductless glands are—1. the suprarenal bodies; 2. the thyroid body; 3. the anterior part of the pituitary body; 4. the carotid and corccygeal glands; and, perhaps, 5. the pineal gland.

I.—*Suprarenal Capsules.*

The suprarenal capsule consists of two parts, a cortical and a medullary. In men the former is usually opaque and yellow, in consequence of the quantity of fat contained in the cells, while the medulla is of a greyish semi-transparent appearance. Between the two is a line of a deep-brown colour, which is sometimes called the intermediate layer, and at this part softening occurs very rapidly after death, so as to cause a separation between the cortical and medullary portions.

The whole organ is enclosed in a fibrous **capsule** which sends processes into the interior. These in the cortical portion enclose spaces which are filled by peculiar **cells,** resembling most closely epithelial cells, but whose real nature is extremely doubtful. In man the cells are roundish or polygonal;

in some of the lower animals, more particularly the dog and horse, they are elongated. They very commonly contain fat in large quantity. Usually the cells fill the space in which they lie, but sometimes indications of a central lumen are present.

The **cortex** is divided into three zones by a difference in the shape of the cell masses. In the outer portion these are roundish or irregularly-shaped groups. This portion is called the **zona glomerulosa.** In the middle portion, which is the thickest of the three zones, they form elongated figures radially disposed. This is called the **zona fasciculata.** In the innermost zone the cells again form irregularly-shaped groups or anastomosing cords. This is called the **zona reticularis.** In man the limit between this and the zona fasciculata is badly marked, although the cells in the former contain much brown pigment. In many animals the zona glomerulosa is absent, the long columnar-shaped cell masses of the fasciculata reaching to the surface of the organ.

The fibrous septa of the cortex are continued into the **medullary portion,** and here are seen as fibres and membranes partially enclosing irregularly-shaped spaces, which communicate freely with one another. These spaces are occupied by cells which still have an epithelial appearance, but differ from those of the cortex in being much more delicate, in never containing fat, and in assuming a brown colour when placed in chromic acid or its salts. They are of very irregular and variable shape, sometimes branched, and have frequently been compared to the cells of a nerve ganglion. It has been found that while the cortical portion of the suprarenal capsules is developed from the mesoblast, the medullary portion is derived from the sympathetic nervous system (Balfour and Mitsukuri). These observations have, however, recently been disputed by Janosik, who finds in mammals and birds that the suprarenal capsule has no immediate connexion with the sympathetic nerve, but is developed close to the ovary or testis, and, like the sexual gland, mainly from the epithelium of the pleuro-peritonaeal cavity. Gottschau, also, denies that the medullary portion of the suprarenal body has any genetic relation with the sympathetic nerve cord. He finds that the cortical portion is developed long before the medulla, and he arrives at the remarkable conclusion that in the mature organ the medulla is continually being destroyed in the formation of a secretion which is discharged into the veins, and is renewed by the inward growth and transformation of the inner portion of the cortex, while a new growth of cortical matter is always taking place at the surface immediately subjacent to the capsule. A perpetual growth thus takes place at the periphery, and a destruction at the centre, and the newly-formed parts pass gradually inwards, helping to form in succession the different layers of the cortex and medulla, until they are transformed into the secretion in the most central portions of the organ.

The suprarenal capsules derive their very abundant **blood supply** from various sources, directly from the aorta, and also from the phrenic and renal arteries. In the cortex the arteries break up into capillaries which surround the cell masses, and whose meshes correspond in shape with those of the latter. Since the greater portion of the cortex is occupied by the radiating zona fasciculata, the most of the vessels run a radial course, connected by numerous cross branches. In the medullary portion the vessels are wider, their walls very thin, and closely surrounded by the medullary cells. From these vessels, which may be looked on as venous capillaries or small veins, vessels with proper coats are formed, and coalesce to form the chief vein, which leaves the organ by its anterior surface.

The **nerves** are very numerous, and consist both of medullated and non-medullated fibres. They form a plexus chiefly in the medullary portion, connected with which are numerous ganglia. The termination of the nerves is unknown.

Klein describes **lymph spaces** between the cells of the cortex, which pass into sinuses between the cells and the fibrous tissue enclosing them. From this the lymph passes into true vessels with valves, which form a plexus in the capsule, and also in the connective tissue about the central vein.

1. Human suprarenal capsule hardened in Müller's fluid, and subsequently in alcohol. Sections perpendicular to the surface; logwood or picrocarmine; balsam.

In balsam preparations the fat will have been removed from the cortical cells by the spirit and oil of cloves.

Sections should be shaken in a test-tube, as was done in the case of the lymph glands. The cells will by this means be partially removed from the spaces in which they lie, and the shape of these spaces will be more evident. The shaken sections should be stained in logwood or picrocarmine, and mounted in glycerine. Some cortical cells will remain, and the fat will be seen in their interior.

Sections of the suprarenal capsules of other animals should be examined. Also injected preparations in which the close relation of the cortical and medullary cells with the blood-vessels will be seen.

II.—*Thyroid Body.*

The **thyroid body** is enclosed in a firm fibrous **capsule.** Processes from this pass into the organ, and divide it into lobes and lobules, and still finer septa bound the **acini** or **alveoli,** which are hollow vesicles, lined by epithelium. They differ considerably in shape and size. They are round, oval, or branched. All have a membrana propria, or basement membrane, and a single layer of epithelial cells, which are columnar or cubical. Every acinus, except the very small ones, has a cavity, or lumen, in its interior. This may be filled with a clear fluid, which coagulates in acetic acid and in alcohol. In adult animals the cavities are very commonly occupied and greatly distended by a viscid, yellowish, so-called colloid material. Fattily-degenerating epithelial cells, cholesterine, oxalate of lime, and blood are sometimes found in the glandular spaces.

The **blood-vessels** are very numerous, and form a close plexus on the outside of the alveoli.

The **lymphatics** commence as sinuses, lying immediately external to the alveoli. They open into lymphatic vessels, which have valves, and which accompany the larger blood-vessels, and are, like them, contained in the connective-tissue septa. Baber has found that the lymphatics frequently contain a fluid of the same character as that which exists in the alveoli. Hence the absorption by the lymphatics of matters formed by the epithelial cells and secreted primarily into the alveoli is probable.

There is little known of the distribution of the **nerves.** They seem to be chiefly for the supply of the vessels. They are provided with ganglia.

The thyroid of a child, hardened in Müller's fluid and alcohol. Sections stained in logwood or picrocarmine; mounted in balsam.

III.—*Pituitary Body.*

The **pituitary body** consists of two parts, which differ completely from one another, both in structure and origin.

The **posterior lobe** is a diverticulum from the floor of the third ventricle of the brain. It is continuous with the infundibulum, and contains nerve fibres, connective tissue, and vessels.

The **anterior lobe** is larger, and is derived from a portion of the epithelium of the pharynx, which is pinched off from its attachment to the latter during fœtal life. It consists of groups of epithelial cells, which are polygonal, granular, and differ a good deal in size. The groups are sometimes completely separated from one another by connective-tissue septa; at others they form elongated masses, which communicate in a sort of network. The fibrous tissue which separates the groups of cells is continuous with the capsule which surrounds both lobes. The blood-vessels and lymphatics run in this fibrous tissue.

Human pituitary body. Hardened in Müller's fluid and alcohol. Logwood or picrocarmine; balsam.

Examine sections through the head of the embryo chick of the sixth or seventh day, to see the origin from the pharynx of the anterior portion of the pituitary body.

IV.—*Coccygeal and Carotid Glands.*

These consist essentially of small arterial vessels, which are here and there dilated into pouches, or form glomeruli. They are surrounded by a thick mass of cells, which have a somewhat epithelioid character. That these organs are glands in any rational sense of the word is more than doubtful.

APPENDIX.

APPENDIX.

I.—MEASURING OF MICROSCOPIC OBJECTS.

OBJECTS are measured by means of an **eye-piece micrometer.** This is a piece of glass with lines ruled on it at equal distances, and which is placed in the eye-piece so as to be in focus, and clearly seen. The lines appear always at the same distance apart, but the value of the intervals between the lines differs with each object-glass, and with each variation in the length of the tube of the microscope. It is best to determine their value once and for all for each object-glass with the tube pushed in and with the tube drawn out to its full extent.

To make the determination a **stage micrometer** is employed. This is a piece of glass on which lines are ruled at a known distance apart, generally $\frac{1}{100}$ mm. The lines on the stage micrometer are brought into focus, and it is seen how many divisions of the eye-piece micrometer correspond to each division of the stage micrometer. Suppose each of the latter is equal to two of the former, then we know that the interval between every two lines of the eye-piece micrometer is equal with that object-glass and that length of tube to $\frac{1}{200}$ mm. The stage micrometer is now removed, and the object to be measured is substituted for it, brought into focus, and observed through the eye-piece provided with its micrometer. The lines of this appear over the object, and we know the value of the interval between the lines. Suppose the object extends over four of these intervals, it then equals $\frac{4}{200}$ or $\frac{1}{50}$ mm.

Once the value of the divisions of the eye-piece micrometer is known for any object-glass and length of tube, further reference to the stage micrometer is unnecessary, but objects may be measured by the eye-piece micrometer, just as ordinary objects are by a foot-rule.

II.—DRAWING MICROSCOPIC OBJECTS.

It is of inestimable value to a microscopist to be able to draw accurately the objects which he sees. It is, however, just as difficult to make microscopic drawings as to draw ordinary objects, and it is not to be supposed that a person with no artistic training will succeed in producing good representations of objects as seen in the microscope.

Microscopic drawings are usually made with the help of an instrument called a **camera lucida,** which consists of one or more prisms fitted to the eye-piece, and so arranged that the object under the microscope and the table at the side of the instrument are seen at the same time. The image of the object appears projected on the table, and if a piece of white paper be placed on this, the outlines of the image can be traced with perfect accuracy. The details must then be filled in with whatever artistic skill the operator may possess.

Some practice is necessary in the use of the camera. The chief point to be attended to is the management of the light. If the object is too strongly illuminated by light from the mirror of the microscope, the point of the pencil cannot be seen, while, if the light on the paper be too intense, the outlines of the image are obscured. It is best to work by artificial light, using, to illuminate the object, an argand gas flame, which can be raised or lowered at will, while a candle placed near the paper will throw sufficient light on the pencil.

III.—DETERMINATION OF THE MAGNIFYING POWER OF THE MICROSCOPE.

A stage micrometer is focused, and by means of a camera lucida the lines are drawn on a piece of paper placed exactly ten inches from the eye. The intervals between the lines thus drawn are measured and divided by the actual intervals between the lines on the micrometer; the quotient gives the magnifying power of the system employed. Suppose the lines on the paper are $\frac{1}{2}$ centimetres apart, while those on the micrometer are $\frac{1}{100}$ millimetre, the magnifying power is 500.

If the paper is placed nearer to the eye than ten inches, the lines on it will be closer to one another, and if further away they will be more widely separated than if the paper is exactly at ten inches. Consequently, in comparing magnifying powers it is always necessary to take the image as it appears at the same distance from the eye. In this country the distance is arbitrarily taken at ten inches, which is about the average distance at which small objects are distinctly seen. On the Continent the distance is usually taken at twenty-five centimetres.

IV.—ENUMERATION OF BLOOD CORPUSCLES.

The **red corpuscles** are so numerous that it would be impossible to count them in undiluted blood. The first step is, consequently, to dilute the blood one or two hundred times. The dilution is usually effected in a graduated pipette, in the expanded part of which is a little free globule of glass, which, as the pipette is moved, falls about in the diluted blood, and helps to distribute the corpuscles evenly through the fluid. This very efficient instrument is known as the **mélangeur Potain.**

The diluting fluid must be of such a kind as not to dissolve the corpuscles, and not to distort them to such a degree as to make the distinction between the red and white corpuscles unrecognisable. A five per cent. solution of sodic sulphate is a good and easily prepared fluid for this purpose.

The diluted blood is next introduced into a **cell,** made by cementing a very thin ring of glass on a slide. This cell has, when covered with a cover-glass, a known capacity, and if the corpuscles contained in it (or a measured portion of it) are counted, and the number obtained multiplied by one or two hundred, according to the dilution, the number of corpuscles in the corresponding volume of blood will be known. To facilitate the counting, the floor of the cell is marked out in squares (Zeiss. Gowers. Hayem).

Sometimes the counting is not made in a cell, but the diluted blood is introduced into an accurately calibrated **capillary tube,** and the corpuscles contained in a known length of this are determined (Malassez). A glass divided into squares is contained in the eye-piece of the microscope, in the position ordinarily occupied by the eye-piece micrometer. A quarter-inch objective is used. A stage micrometer is placed under the microscope, and the tube of the instrument is drawn out until one side of the squared space in the eye-piece exactly corresponds to $\frac{1}{2}$ mm. The counting must always be made with the same eye-piece and object-glass, and with the same length of tube. When once this length has been determined, a mark should be made on the draw-tube, by which it can always be directly arrived at without the use of the micrometer.

The capillary tube containing the diluted blood is now substituted for the micrometer, and the number of corpuscles contained in that length of it which is covered by the squaring in the eye-piece (that is in $\frac{1}{2}$ mm.) is counted. The capacity of this length of tube is different in each instrument, and is marked on the piece of glass on which the capillary is cemented. It is always a small fraction of a cubic millimetre. The number of corpuscles got is multiplied, first by the denominator of this fraction; then by one or two hundred, according to the original dilution, and the products give the number of corpuscles in a cubic millimetre of blood. The capillary tube has the disadvantage of requiring the employment always of the same microscope, which must be provided with a draw-tube.

When the **white corpuscles** are to be counted, it is best to dilute the blood only ten times, and to use a fluid which dissolves the red corpuscles, but leaves the white. Such a fluid is water containing one-third per cent. glacial acetic acid (Thoma).

Several countings should always be made of the same blood, and the mean of these taken. As the accuracy of the graduation of the instruments is never to be depended on, it is necessary to make first, for each instrument, a number of countings of the corpuscles in healthy blood taken from different individuals. In this way a standard is got, with which subsequent results can be compared.

V.—CIRCULATION OF THE BLOOD.

In order to see the circulation of the blood, it is necessary to examine some transparent part in a living animal. The web of the frog's foot is the part most commonly chosen, and although not so good in many respects as the lung or mesentery of the same animal, yet the facility with which the examination can be made without inflicting any pain on the animal justifies the choice.

A thin piece of board, sufficiently large to support the body of the frog, is taken, and in one end of it a V-shaped notch is cut, about the size of the interval between two of the toes of the hind foot. The frog is then made motionless, by the injection under the skin of the back of one drop of a one per cent. solution of curara. This poisoning gives no pain, and is recovered from in a few hours. The frog, when paralysed, is placed on the board. Soft threads are tied to two of the toes, and the intervening portion of the web is gently stretched over the V-shaped notch. The threads may be fastened with pins, or fixed by being drawn through slits in the board. Great care must be taken not to overstretch the membrane, and so interfere with the natural movement of the blood.

The board is placed on the stage of the microscope, and is fixed with the clips in such a position that the web lies over the hole. The web is examined first with the low power, and then with a stronger object-glass.

During the examination the animal should be kept covered with wet blotting-paper or lint, and a small quantity of water should frequently be applied to the web.

In the **arteries** the flow is so rapid, that the individual corpuscles cannot be seen. The current is not uniform, but experiences an acceleration at each systole of the ventricle. The corpuscles move in the axis of the vessel, separated from its sides by an interval occupied only by transparent plasma. In this, white corpuscles are seldom seen.

In the **capillaries** the current is much slower, so that the corpuscles can be readily seen. It will be noticed that, owing to the narrowness of the vessels, they can move only in single file, and that often they have to alter their shape as they squeeze themselves through the narrow and tortuous channels. Once the obstruction is passed, their normal shape is at once regained. Thus the flexibility and elasticity of the corpuscles is demonstrated. In the network of capillaries the flow is from the arteries to the veins, but in any particular vessel it will be seen that the direction of the current is not constant, but the blood may become stagnant for a short time, or may flow sometimes in one direction, sometimes in the other. In the capillaries the blood corpuscles, both red and white, come into contact with the wall of the vessels.

In the **veins**, which are more capacious than the arteries, the current again becomes rapid, but does not reach the velocity in the arteries. The corpuscles can be more or less distinctly seen according to the rate of flow of the blood. The current is continuous. The mass of the corpuscles moves in the axis of the vessel, and in the clear plasma in contact with the wall numerous white corpuscles can be seen rolling along slowly, and sometimes adhering for a time to the interior of the vein and coming to rest. The slower the circulation, the more numerous are the white corpuscles in the peripheral layer of plasma.

VI.—INJECTION OF BLOOD-VESSELS.

With rare exceptions, the **injection masses** which are now employed consist of gelatine, coloured either with soluble Prussian blue or with carmine.

Soluble Prussian blue is prepared by precipitating ferrocyanide of potassium with ferric chloride. The salts are dissolved separately in a nearly saturated solution of sodic sulphate, and the ferric chloride is poured into the ferrocyanide of potassium. The precipitate is collected on a filter, and washed with water until the soda salt is removed, and the blue mass begins to come through the filter. The washing is then discontinued, and the contents of the filter are dried and preserved for use. Soluble Prussian blue can be bought from the druggists. Its preparation is troublesome and tedious, and need not be undertaken unless large quantities are required.

To prepare the blue injection mass, 10 grams of the finest gelatine are placed for some hours in cold water. The water which has not been absorbed is poured off, and the swollen gelatine is placed in a beaker, which is heated in a water-bath until the gelatine is dissolved. There is then added slowly, and with constant stirring, 50 cc. of a two per cent. solution of soluble Prussian blue, previously filtered and warmed. When the mixture is completed, the mass is strained through fine new flannel, and is then ready for use.

The carmine injection mass is more difficult to prepare, and is consequently less employed than the mass of Prussian blue. Four grams of carmine are dissolved in 8 cc. strong liquor ammoniæ, and diluted with 50 cc. water. The solution is filtered. The filtrate is warmed, and there is slowly added to it a solution of gelatine, made as above directed, and in the same quantity. To this mixture, while it is still kept warm in the water-bath, there is added, drop by drop, a weak solution of acetic acid. At a certain point the smell of ammonia disappears, and the colour of the mass changes from dark purple red to bright crimson. This point is difficult to attain exactly. If enough acetic acid is not added, the soluble carminate of ammonia diffuses through the walls of the vessels, and stains the tissues. If the acetic acid be in excess, the carmine is precipitated in coarse granules, which will not pass through the fine vessels. When the right point is hit, the carmine is precipitated, but in such fine particles, that they are invisible even in the microscope.

It is best to prepare the injection masses immediately before they are used, but they can be kept for some time if chloral be added to the gelatine (Hoyer). Before being used, however, the mass must always be carefully filtered through fine flannel.

The injection may be made with a **syringe,** or by the method of constant pressure. The former method is the less troublesome, and with some practice gives sufficiently good results. Injection syringes can be bought at the instrument makers. Each syringe should have several cannulæ of different sizes to go into the vessel, and the junction of the cannula with the syringe should be by an intermediate piece, in which there should be a stop-cock. As it is of great importance that no air should be introduced into the vessels, it is best to fill the cannula with pure glycerine before tying it in the artery. The injection mass is kept fluid in a water-bath heated to about 104° F. The syringe should be heated to the same temperature, as should the body of the animal, or the part to be injected. In general it is best to kill the animal by chloroform, and proceed at once to the injection while the body is still warm; but if the animal or part has been for some time dead, or if the injection is likely to occupy any considerable time, it is well to immerse the object to be injected in water heated to 104° F.

The syringe is carefully filled with the injection mass, so as to exclude all bubbles, the intermediate part put on, the piston slightly pushed down, so as to fill this and to force some of the mass into the free end of the cannula. Then the connexion with the cannula is made, and the piston pushed down as slowly and as steadily as possible. When the injection begins to flow out from the veins unmixed with blood, a ligature is placed on the points of exit, and the pressure continued so as fully to distend the vessels. When this is accomplished, the stop-cock of the intermediate piece is turned, and, leaving this in connexion with the cannula, the syringe is removed. The injected parts are allowed to cool, and are then placed first in weak, and subsequently in strong alcohol, until they are sufficiently hard to allow of sections being made.

In the method of injecting by **constant pressure,** the injection mass is contained in a wide-mouthed bottle, closed with an india-rubber cork, through which two glass tubes pass.

One of these reaches to near the bottom of the bottle, and consequently dips into the injection mass. Its outer end is bent, and has fixed on it an india-rubber tube, which at the other end is provided with a strong clip, or, better, has attached to it the intermediate piece of an injection syringe, with its stop-cock, and which fits into the cannula in the artery. The second glass tube passes just through the cork of the bottle which contains the injection mass, and terminates above the level of the latter. Its outer end is connected by an india-rubber tube with the second part of the apparatus or pressure bottle. This consists of a large Woulfe's bottle, with three necks. Into each of these fits an india-rubber cork, perforated by a glass tube. One of these tubes communicates with the injection bottle, a second with a mercurial manometer, and the third with a reservoir which contains water, and which can be raised to any desired height by means of a cord passing over a pulley in the ceiling. It is clear that, when the reservoir is raised, the water will flow into the pressure bottle, and compress the air contained in it. This pressure is transmitted to the surface of the injection, which is by this means forced out into the tube in connexion with the cannula. The greater the height to which the reservoir is raised, the greater is the pressure exerted. The exact amount of this can be determined by the manometer which is in connexion with the pressure bottle.

The cannula having been suitably fixed in the artery, the bottle containing the melted injection mass should be placed in the water-bath, and the reservoir raised so as to drive out the air contained in the tube going to the cannula. When the mass flows freely, the connexion with the cannula is made, taking great care that no air is admitted. The pressure is then raised, first to about two inches of mercury, subsequently to from five to eight inches. When the mass flows freely from the veins, these are tied, the pressure continued for a short time so as to completely fill the vessels, the stop-cock attached to the cannula closed, the reservoir lowered, and the injection bottle detached from the stop-cock. The subsequent treatment of the injected parts is the same as when the syringe has been used.

The pressure bottle may be put in connexion with a water-tap, and the pressure got from this source, instead of from the moveable reservoir. It is difficult, however, in this way to maintain a perfectly even pressure.

It is difficult to make good injections, and the necessary skill can be acquired only by practice. Most failures are due to the use of a bad mass. There are some gelatines which cause a precipitate in the Prussian blue solution. These should be rejected. The finest French gelatine, which is sold in thin sheets, is the best. The greatest care should be taken to have all parts of the apparatus clean. This is more easily accomplished when the syringe is made of glass than when it is, as is usual in this country, made of metal.

Even with every precaution, it is not possible always to reckon with certainty on a successful result.

VII.—ON HARDENING TISSUES.

Most tissues and organs are, in their fresh state, of such a consistence that thin sections of them cannot be made by a razor or microtome. If it is desired to have sections of a fresh object, before it has been acted on by reagents, this may be accomplished by freezing the part to be cut, when the sections may be made by a razor or in the freezing microtome, or the perfectly fresh tissue may be cut by **Valentin's knife,** which is a knife with two parallel blades, whose distance can be regulated by a screw. The sections made by this instrument are seldom thin enough to allow of examination by high powers, but they are often useful, particularly in pathological investigations, when it is desirable to see the distribution of changes which are often obscured by the action of alcohol, such as fatty or pigmentary deposition, hæmorrhage, &c. Sections of fresh tissues must be examined in salt solution, and cannot be preserved.

In most cases it is necessary to harden a part prior to its examination. After hardening, the part should not only be increased in consistence as a whole, but the inequality which exists in the consistence of the different tissues, when in the fresh condition, should be equalised, so that the entire mass should have an uniform density.

The following are the hardening reagents which are most commonly employed :—

1. **Alcohol.**—For many objects methylated spirit answers very well. It should be 'redrawn,' perfectly colourless, and miscible with water in all proportions without turbidity.

The object to be hardened should be of small size, and a considerable quantity, at least twenty times its bulk, of the spirit should be used. Here, as in all other methods of hardening, the object should not lie on the bottom of the vessel, but should be suspended by a thread near the surface of the hardening fluid. In this way it is completely surrounded by the fluid, and the water and other matters which exude from it sink away from it to the bottom of the vessel.

The spirit should be changed at the end of twenty-four hours, and again on the third day if the object is not then sufficiently hard. The consistence can be readily ascertained by touch. It is often useful in hardening membranous structures to pin them out on a piece of wood or cork, to prevent them from falling into folds.

For very delicate objects the alcohol should be at first diluted with two parts of water, and the strength gradually increased. To complete the hardening it is desirable to use absolute alcohol, but its great expense prevents its very general employment.

Objects hardened in alcohol stain well in most dyes.

Acinose glands, as salivary glands and pancreas, the walls of the digestive tube, and the testicles, harden very well in alcohol.

2. **Chromic acid,** used in watery solution from $\frac{1}{4} - \frac{1}{8}$ per cent., will not harden large objects, as its power of penetrating the tissues is very limited. A relatively very large quantity of the solution should be used. It should be changed after twenty-four hours, and then every second day for a week or ten days. Chromic acid will not harden by itself. It equalises the consistence of the different tissues, but the final hardening must be accomplished by alcohol. After removal from the acid, the object should be well washed, which is best

done by suspending it in a large vessel in which the water is frequently renewed. When it ceases to give a yellow colour to the water it is placed in alcohol, which should be changed two or three times at intervals of twenty-four hours. A muddy precipitate commonly forms in the alcohol after the object has been placed in it. This does not occur if the vessel is kept in the dark. Whenever it does form, the alcohol must be changed.

Objects which have been in chromic acid stain with difficulty.

Nerve centres, nerves, epithelial organs, harden well in chromic acid.

A mixture of **chromic acid and spirit** has been much used of late as a hardening agent. It may be made by slowly adding 180 cc. of methylated spirit to 20 cc. of a 5 per cent. solution of chromic acid. This is recommended by Rutherford for hardening the retina and cochlea. It should be changed after twenty-four hours, and then every second day for ten days, when the object should be transferred to pure methylated spirit.

A mixture of one part of methylated spirit and two of a $\frac{1}{6}$ per cent. solution of chromic acid is much praised by Klein as a general hardening agent, and gives excellent results. The object should remain in it from one to three days, and should then be placed in dilute alcohol which should be gradually increased in strength. Mixtures of chromic acid and spirit should be made only when they are wanted, and should be kept in the dark.

3. **Bichromate of potash** may be used alone in 2 per cent. solution, but is more commonly employed as **Müller's fluid,** consisting of

Bichromate of potash, $2\frac{1}{2}$,
Sulphate of soda, 1.
Water, 100.

Müller's fluid has great penetrating powers, and will consequently harden objects of considerable size and density. An entire human kidney or even a whole brain may be hardened if the fluid is used in sufficient quantity and changed with sufficient frequency.

Müller's fluid hardens very slowly. It should be used in large quantities and changed within the first twelve hours, then every second day for a week, and then once a-week until the hardening is completed. This will require a month or six weeks at least. The object should then be washed in water as long as it continues to give up colour, and then placed in alcohol, which should be changed as long as a turbidity forms in it. In many cases the hardening may be completed by Müller's fluid, and the object need not be put into alcohol prior to the examination. Hardening in Müller's fluid alone should be employed when it is desired to see fat in the interior of cells. The sections should be stained in osmic acid, which blackens the fat, and also makes the cellular elements very distinct. This method is very useful for the mammary gland, developing spinal cord, and in pathological work for fatty liver, kidney, degenerated nerve centres, &c. Such sections should be mounted in glycerine or Farrant's solution. Müller's fluid is most useful for the nerve centres, the retina, and for parenchymatous organs, as the liver, kidney, ovary, testicle, &c. Objects hardened in it do not stain so readily as those which have been in alcohol only, but better than those which have been hardened in chromic acid.

Bichromate of potash enters into the composition of **Erlicki's fluid,** which has been again recently recommended by Weigert for hardening the nerve centres. It consists of

Bichromate of potash, 2½.
Sulphate of copper, ½.
Water, 100.

It hardens more quickly than Müller's fluid. It gives the spinal cord a good consistence in ten days, and if the hardening be carried out at the temperature of 104° F. it is completed in four days. The fluid should be changed every day. It is for the purposes mentioned an excellent mixture.

4. **Bichromate of ammonia,** in 2 per cent. solution, for hardening the nerve-centres. Takes about a month. It is employed in the same way as Müller's fluid. It is much more soluble than bichromate of potash, and can be more easily

washed out of the tissues. Beyond this it does not seem to have much advantage over the much cheaper potash salt.

5. **Neutral chromate of ammonia.**—In 5 per cent. solution recommended by Heidenhain for hardening the kidney. It preserves the structure of the epithelial cells better than the bichromates. The object is left in it for twenty-four to forty-eight hours, then carefully washed in distilled water, and the hardening completed in alcohol.

6. **Picric acid** may be used in saturated watery solution, or better in **Kleinenberg's solution,** which is prepared as follows:—

Cold saturated solution of picric acid in water, 100 cc.
Add 2 cc. strong sulphuric acid ; filter.
Add to filtrate 300 cc. distilled water.

Objects to be hardened in picric acid must be small, as the acid does not penetrate; they should be left in the acid seven to twelve hours, and then transferred to dilute spirit, which should be gradually increased in strength, and changed so long as it takes up a yellow colour from the object.

Picric acid is sometimes used to decalcify small fragments of bone or teeth. In these cases the object must be left in the solution much longer than when mere hardening is required, and there should always be some undissolved acid at the bottom of the vessel, so that the concentration of the solution may be kept up.

Picric acid stains the tissues yellow, particularly horny and elastic tissues. It does not interfere with logwood or carmine staining.

7. **Osmic acid,** 1 per cent. solution, hardens only superficially; the object therefore must be small. Requires twenty-four to forty-eight hours. Blackens fat and nerve fibres. Invaluable for organs of special sense, as retina, cochlea, taste-buds, &c.

A mixture of **chromic acid $\frac{1}{4}$ and osmic acid $\frac{1}{10}$ per cent.** gives admirable results in the case of many delicate organs, as the retina. Objects hardened in it may be mounted in balsam without being stained, as the elements are very distinct.

After the prolonged action of osmic acid, objects will not stain in picrocarmine.

8. **Nitric acid,** 4 to 5 per cent. of the strong acid, in water. Useful for very delicate objects, as young embryos, and retina. The object must remain ten to twenty minutes in the acid, and be then placed in strong alcohol.

VIII.—ON EMBEDDING.

In many cases, where objects which have to be cut are so small or so delicate that they cannot be held in the hand, it is necessary to embed them in some substance which will give them the required bulk and support. These substances are of such a nature that they are fluid when the object is placed in them, and subsequently become solid.

The embedding mass most commonly employed is made of equal weights of **white wax and olive oil** melted together in a capsule over a water bath. In winter it is necessary to add a little more oil, and in summer a little more wax, so as to have at all temperatures a mass of equal consistence. The mass should be raised to a temperature just sufficient to melt it, and great care must be taken not to have it too hot, in which case the object would be injured by the heat, and the too fluid mass would soak into the interstices of the tissues, from which it could not subsequently be removed.

From a piece of stiff writing-paper a mould or box is made to contain the embedding mass. The box should be oblong, and each of its long sides should be of the same size as the bottom, so that the cross section of the box is a square. Its dimensions must of course vary with those of the object; but as the latter is usually small, the box may be made of such a size that while it supports the object it can be held conveniently in the hand. A good size is 2 inches long by $\frac{7}{8}$ inch broad. The piece of paper is taken $3\frac{6}{8}$ by $2\frac{5}{8}$ inches. It is folded in length into three equal parts, each $\frac{7}{8}$ inch broad. It is then folded tranversely $\frac{7}{8}$ inch from each extremity. Thus it is divided in the middle into three parallelograms, 2 inches by $\frac{7}{8}$ inch, and at each end into three squares, each $\frac{7}{8}$ inch. The four corner squares are now folded along the diagonal from the corner of the paper inwards. The two lateral parallelograms are turned up to form the sides of the box, while the central parallelogram forms the floor. The central square at each extremity forms one of the ends of the box, while the corner squares, folded as directed, form flaps, which are turned in and fastened with pins, which should be inserted as close as possible to the upper edge of the box.

The box is placed on a small piece of flat board, and the melted mass is poured into it.

The object to be embedded must always come out of spirit, since the wax mass will not adhere to a surface wetted with water. The excess of spirit is removed from it with filter-paper. It is transfixed with a needle, whose point must project a quarter of an inch or so from its surface. It is plunged into the melted mass contained in the box, and the needle passed through the bottom of the latter and fixed into the board beneath. By this means the object is held in proper position while the mass is hardening. It is most important that this position should be rightly chosen. The object must be placed as close as possible to one end of the box, and great care must be taken that it does not rest on the bottom of the box, but that it is surrounded on all sides by the mass. As the object is concealed from view when the mass has hardened, its position in the box must be noted, in order that the sections may be made in the right direction. It is a good rule to embed objects in such a position that sections made in a plane at right angles to the long axis of the box will cut the object in the desired direction. When the mass has cooled the needle is withdrawn, having first been slightly turned on itself, as is done before withdrawing a hare-lip pin. The paper mould is removed. The hardened block is held in the hand, and with a razor, moistened in spirit, the wax is cut away at the end at which the embedding was made, until the object is exposed. If this is small and the quantity of wax about it is excessive, the edges of the block may be pared away, leaving just enough wax to support the object. Sections are then made through the object and the supporting mass, as was directed in p. 77, and are received into a watch-glass or capsule containing clean spirit. The adhering wax can be removed with needles, when the sections may be stained and mounted in any of the usual ways.

Once the block has been cut, it cannot be left exposed to the air, as the object would dry and be spoiled, but it may be placed in spirit and preserved for future use. The parings of the mass and the old blocks may of course be melted over again.

Besides the mass of wax and oil, other substances are employed for embedding objects prior to making sections.

The wax and oil mass should adhere only to the surface of the object embedded in it, and should not penetrate into the interstices or hollows, from which it could not be removed. Now, many tissues or organs are so delicate and soft that the support afforded by the wax and oil would not suffice to allow of their being cut. In these cases it is advantageous to use some material which soaks through the object, and which, when hardened, gives enough support and consistence to admit of fine sections being made. Of such masses, the principal are gum, paraffin, collodion, and albumen.

2. **Gum.**—The object, which must be free from alcohol, is placed for some hours in a syrupy solution of gum arabic. It is then placed in alcohol, which coagulates and hardens the gum. The object embedded in the gum may then be cut directly, or, if it is not of sufficient size to hold, may be further embedded, gum and all, in wax and oil. In many cases, if the object be very small, it may be fixed to a piece of alder pith by a drop of strong gum, and then placed in alcohol. After hardening, the sections may be made. If the alcohol is too strong, the gum is made so hard that it takes the edge off the razor. The suitable strength of the spirit can be determined only by trial. If the gum is too hard, the mass should be placed for some time in more dilute alcohol. The sections, when made, are placed in tepid distilled water, which dissolves out the gum; then stained, and mounted in one or other of the usual ways. This method is very ready, and in many cases useful, but is inferior to those which are next to be described.

3. **Paraffin.**—A paraffin should be chosen which melts below 110° F. The object to be embedded must be small. It should be stained *en masse*, washed in water, and thoroughly dehydrated in strong alcohol. It is then placed in oil of cloves until it is soaked through by this fluid. It is next transferred for a short time to oil of turpentine. It is then placed in the melted paraffin, which should be kept a few degrees above its melting point in a drying oven provided with a gas regulator. According to its size, the object should remain in the paraffin for from half an hour to twenty-four hours. It should be completely permeated by the paraffin. It is then placed in a paper box and the melted paraffin poured about it, as was done in the case of the wax and oil, or it may be removed from the paraffin and kept for any length of time until it is required for section. Being completely impregnated with the paraffin, there is no risk of injury from drying. The sections may be made with a dry razor, or may be cut in a microtome. If a suitable instrument of the latter kind be used, sections $\frac{1}{6000}$ inch in thickness can be readily made. The sections are placed on a slide, which is gently warmed over a lamp until the paraffin melts; the melted paraffin is washed away by turpentine dropped on the section. The preparation may then be permanently mounted in Canada balsam. This method, which is universally used in embryological and comparative anatomical work, has more limited application in ordinary histology. It is, however, of great service in the examination of such objects as the lung, testicle, retina, spleen, and other parts of great delicacy, whose parts have only a slight cohesiveness. In the latter case it is well to cement the sections to the slide before dissolving out the paraffin, as will be explained further on, when speaking of the method of mounting sections in series.

Other masses may be used in somewhat the same way as paraffin. The object, after having been in oil of cloves, may be soaked in melted **cacao butter.** The sections are made with a razor moistened with oil of cloves, and the embedding mass can be dissolved out by the same fluid. Or a mass composed of **spermaceti 4, castor oil 1,** may be employed. The object, prior to being placed in this, is stained, dehydrated, and soaked through with oil of bergamot. The sections are cut with a razor wetted with olive oil, and the embedding mass is dissolved out by a mixture of oil of turpentine 4, creasote 1.

4. **Collodion** (Duval).—Objects need not be stained prior to being embedded in collodion. The object is placed in strong alcohol until all water is removed, then placed for about half an hour in ether, and then in collodion for from ten minutes to twenty-four hours. It is then, with the adherent collodion, dropped into ordinary alcohol (methylated spirit). This coagulates the collodion, which, however, retains a perfect transparency, and has a very suitable consistence for cutting. The sections may be made with a razor, or in a microtome, the knife being wetted with spirit. They may be stained in carmine or logwood, since the adherent collodion does not stain, or only very feebly. The collodion holds the parts of the object together, and does not interfere with the examination, since it remains transparent. The sections may be mounted in glycerine or Farrant's solution. Oil of cloves dissolves the collodion. If this is no objection, the sections may be mounted in balsam. Oil of bergamot does not dissolve collodion, and may be used as a clarifying fluid instead of oil of cloves.

As collodion has not a sufficient consistence for many objects, it has been recently proposed to substitute for it a solution of **celloidin** (Schiefferdecker). This substance is manufactured by Schering of Berlin, and is sold in small tablets. It is a hard, semitransparent, colourless material. It should be cut into small pieces, and dissolved in a mixture of equal volumes of absolute alcohol and ether. The solution should have a thick syrupy consistence. The object, after being treated successively with alcohol and ether, is placed in the celloidin solution, and left there until it is completely soaked through by it. It is then placed in ordinary alcohol, which coagulates the celloidin and gives the whole a fit consistence for cutting. Absolute alcohol must not be used, as it dissolves celloidin. A very convenient plan is to place the object, after removal from the celloidin, on the surface of a suitably-shaped piece of cork, in a drop of the celloidin, and then to place the cork, with the object attached, in alcohol. After hardening, the cork allows of the object being held in the hand or fixed in a microtome. The sections may be stained and mounted in glycerine, or cleared with oil of bergamot and mounted in balsam. The celloidin need not be dissolved out, as it does not stain, and remains quite transparent. By this method good sections of such organs as the testicle can be got. The parts of the thin sections do not fall asunder, but are held together by the embedding mass.

5. **Albumen.**—The albumen of an egg is mixed with $\frac{1}{15}$ its volume of a 10 per cent. solution of carbonate of soda. The yolk is then added, and the whole well shaken together. The mass is poured into a paper box, and the object, which must be free from alcohol, is placed in it according to the rules given when describing the use of the wax and oil. The box is then placed in a capsule and surrounded, but not covered, with alcohol of about 80 per cent. The capsule is covered with a bell glass, and heated in a water bath to about 167° F. for half an hour. By this time the mass is coagulated on the surface. The box is then placed in alcohol to complete the hardening. The sections are cut and mounted in the usual way (Calberla).

IX.—MICROTOMES.

Although it is of great advantage to be able to cut sections by hand, yet there are few histologists now who depend solely on this method, and who do not, for finer and more accurate work, employ some form of microtome.

Ranvier's microtome represents the most simple form of instrument. It consists of a metal tube, closed below, but open above. Around the upper opening is a flat plate of metal, which supports the knife in making the sections. Through the closed bottom of the tube there passes a screw, by which the object can be gradually pushed upwards in the tube. The object is fixed in the tube either by having wax and oil poured around it, or, better, by being wedged in by dry and compressed alder pith. This, when moistened with spirit, swells, and holds the object firmly in position. By a slight movement of the screw the object is pushed up above the level of the top of the tube, and the projecting part is sliced off with a razor, which glides on, and is supported in its movements by the plate of metal. The capabilities of this instrument are very limited.

A much better form of hand microtome is that of **Schiefferdecker.** In this the object, either embedded or not, is clamped into a tube, and instead of being pushed up through this, as in most microtomes, the plate on which the knife moves is gradually lowered by a screw. The circumference of the plate is graduated, and provided with an index, by which the thickness of the sections can be regulated.

This is, for many purposes, a very handy and efficient instrument. It is particularly useful for cutting sections of the spinal cord.

A much more perfect microtome is that of **Thoma,** made by Jung, of Heidelberg. In it the knife is not held in the hand, but is fixed in a support, which slides in a horizontal path in the heavy metal frame of the instrument. The object, embedded in paraffin, or fixed to a cork with gum or celloidin, is held in a kind of clamp, which is attached to a support which slides in an obliquely ascending path in the frame. As the object rises in its path, its upper surface comes to lie above the level of the edge of the knife, which is drawn across the object, and removes a slice from it. The object is then raised further in its path, and the process repeated. The movement of the object is effected with great accuracy by means of a graduated micrometer screw. If the object is embedded in paraffin, the knife is kept dry, and should be so fixed that its edge is at right angles to the line of its movement. If the object is embedded in wax and oil, albumen, celloidin, or simply fixed on a piece of cork, the knife should be wetted with spirit, and the edge should meet the object very obliquely.

This is a very beautifully-constructed instrument; and if the object is small, and is suitably hardened and embedded, sections may be cut of .003 mm. in thickness, and much larger sections of .01 mm. can be made without difficulty or failure. It is necessary, however, that the hardening of the object should be very perfect, as imperfectly hardened objects yield before the knife.

For general use there is no class of microtome so satisfactory as that in which the object is frozen, and cut when in this condition. Rutherford's **freezing microtome** long enjoyed a well-deserved popularity, but it has now very generally given way to the much more convenient instrument of Williams, made by Swift, of Tottenham Court-road, London.

This consists of a wooden tub, with a lid formed of a glass plate. The plate is perforated in the centre, and through the perforation there passes the upper end of a pillar of brass, which is fixed at its lower end by an expanded base to the bottom of the tub. On the top of the brass pillar is a small table of brass, on which rests the object to be cut. The tub is filled with a freezing mixture of finely pounded ice and salt. This is packed about the central pillar, and soon reduces its temperature to such a degree, that any object placed on the table at the top freezes instantaneously. The lid is applied and fastened on by a screw. The knife is fixed in a metal frame, which rests on three screws. These slide on the glass lid of the tub. Two of the screws are behind the knife, one in front. By turning this latter screw the edge of the knife can be gradually lowered.

The object to be cut must be quite free from alcohol. If it has been in this fluid, it must be placed for twenty-four hours in water. It is then placed in thick gum, in which it must lie until it is completely permeated by it. If the object is of a loose and spongy structure, such as the lung or testicle, a few hours will suffice; but if it has a dense consistence, as the liver, kidney, or nerve centres, it must remain some days in the gum. It is best to cut the object into thin pieces before placing it in the gum. From a piece one-sixth of an inch thick a very large number of sections may be made. When the object is soaked through with the gum, it is placed on the table of the microtome, to which it freezes fast in a very few seconds. The knife is then lowered until its edge touches the upper surface of the object, from which a thin slice is removed. By gradually lowering the front screw the edge of the knife is depressed, and after each depression the knife is pushed across the object, and a section planed off. With a little practice the instrument can be worked with great rapidity, so that one hundred sections per minute can be made. It is not necessary to remove the sections as each is cut; they accumulate in a heap on the upper surface of the blade of the knife, from which they can be transferred by a camel's hair brush to tepid distilled water, where they must lie until the gum is washed out of them. They can then be at once stained and mounted in the usual way, or they may be kept in dilute spirit, or glycerine and water, until they are required. Gum is used because it has, when frozen, the consistence of very firm cheese, and cuts evenly and without injury to the knife. Water, on the other hand, is brittle when frozen, and breaks before the knife, whose edge, too, is injured by the hard spicules of ice. By the method of freezing, objects may be cut whose hardening is not sufficiently perfect to allow of sections being made in the other forms of microtome, and even fresh tissues can be, by this means, successfully prepared for examination.

A modification of Williams's microtome, in which the freezing is effected by ether spray instead of by ice and salt, has been devised by Mr. Groves of King's College. For private work, where only a few sections are required, it is very useful; but for cutting large numbers of sections, such as are required for class demonstrations, Williams's microtome, in its original form, is unrivalled for convenience and rapidity.

X.—ON CUTTING SERIES OF SECTIONS.

It is sometimes necessary to cut the whole of an object into sections of a certain thickness, and to preserve all the sections. In order to accomplish this, a microtome must be employed, and one such as that of Schiefferdecker or of Thoma, which enables the thickness of the sections to be accurately determined. Such serial sections are absolutely essential in embryological research, but are not very commonly required in ordinary histological work. In some cases, however, they are very advantageous.

Thus, a series of sections through the retina, made parallel to its surfaces, are in the highest degree instructive.

When such series are to be preserved, it is of great convenience to be able to mount several of them in order under one cover-glass. In this way a multiplication of slides is avoided, and the different sections, lying side by side in the same preparation, can be readily compared with one another. If the sections were simply placed on the slide, and mounted in the usual way, they would infallibly undergo displacement in the application of the cover-glass, their order would be lost, they would get one over the other, and some would probably float from under the cover-glass and be lost. Hence, in order that a series of sections shall be properly mounted, it is essential that the sections shall be cemented to the slide in their right positions previous to the application of the cover-glass. This may be done in either of the following ways—in both cases the object is supposed to have been embedded in paraffin prior to the making of the sections:—

1. A slide is covered with a very thin layer of a solution of bleached shellac in alcohol, which is allowed to dry. The part of the slide on which the preparation is to be made is painted with an exceedingly thin layer of anhydrous creasote, which dissolves the shellac, and forms a sticky surface. On this the sections are placed in order as they are cut. When as many sections as it is desired to mount on one slide are duly arranged in position, the slide is placed for half an hour or so in a drying oven, at a temperature a few degrees above the melting point of the paraffin. The heat melts the paraffin and evaporates the creasote, and leaves the sections firmly adherent to the slide. While the paraffin is melted, a stream of turpentine is poured over the slide: this washes away the paraffin. A cover-glass on which some Canada balsam has been placed is then applied (Giesbrecht).

This method does not readily admit of the sections being stained after they are cemented to the slide, or of their being mounted in glycerine, as they cannot be washed with alcohol, which would dissolve the shellac cement. On these accounts the following method, being of more general application, is to be preferred:—

The slide is painted with a thin layer of a mixture of collodion and oil of cloves, in the proportion of one to four. On this the sections are arranged. The slide is then placed in the drying oven, to melt the paraffin and evaporate the oil of cloves. If it is desired to mount the sections in balsam, the melted paraffin is washed away with turpentine, and the cover-glass with the balsam at once applied. But if the preparation is to be put up in glycerine, the slide is washed first in turpentine, then in chloroform, then in alcohol, and then in water. The sections may then be mounted in glycerine, or may first be stained with logwood, carmine, or any other staining fluid. If, after staining, it is desired to mount the preparation in balsam, the slide can be treated in succession with alcohol to dehydrate, oil of bergamot, and balsam. Oil of cloves must not be used, as it dissolves the collodion cement (Schällibaum).

By either of these methods upwards of a hundred sections can, if sufficiently small, be mounted under one cover-glass.

Although, as has been said, serial sections are rarely requisite in the ordinary course of histological work, yet when single sections are ragged, or very delicate, or consist of detached parts, whose relative positions it is important to preserve, it will be found highly advantageous to cement them to the slide; and when they are so fixed the processes of staining and mounting can be carried out with a facility and certainty of success otherwise impossible. These methods, which constitute some of the most important of the recent advances in microscopical technique, are peculiarly applicable to the retina, testicle, spleen, and to some pathological objects.

XI.—ON MOUNTING PREPARATIONS.

Objects are seldom mounted **dry.** This is, however, sometimes done, either when, as in the case of blood corpuscles, the object is injured by most reagents, or when, as in the case of the fibrils of white fibrous tissue, it is so transparent as to be invisible, except in a medium of very low density. In such cases the cover-glass may be fastened down by strips of gummed paper applied over its edge, or a rim of melted paraffin may be drawn around it with a heated wire. Fluid cements cannot be used, as they would run in under the cover-glass by capillarity.

In general, objects are mounted either in glycerine or in Canada balsam. In the former case it is not necessary to remove the water from the object, since glycerine mixes perfectly with water; but previous to mounting in balsam the object must be dehydrated, and permeated with some essential oil, with which the balsam can mix without turbidity.

In mounting in **glycerine** the object, either stained or not, is placed in a suitable position on the slide. A drop of glycerine is then placed on the under side of the cover-glass, which is cautiously applied to the object, great care being taken that this is not displaced, that no air-bubbles are included, and that no glycerine gets on the upper surface of the cover-glass. With two pieces of filter-paper applied at opposite edges of the cover-glass any excess of glycerine is removed, until the cover-glass adheres slightly to the slide. The slide is then wiped perfectly dry up to the edge of the cover-glass, and a rim of cement is painted on.

In glycerine preparations many fine details can be seen, which are lost in preparations mounted in balsam, owing to the high refractive power of the latter. It is, however, much more difficult to mount in glycerine than in balsam. The cover-glass is apt to become displaced in applying the cement. The cement often runs in and spoils the preparation, and the glycerine often leaks through the cement. These difficulties may, however, be overcome by practice. If a preparation is found leaking, a stream of water should be poured over it, and after it is carefully dried a fresh layer of cement should be put on.

Many of the advantages of glycerine mounting, without its difficulties, may be had by using **Farrant's solution.** This consists of a mixture of equal parts of glycerine and a saturated solution of arsenious acid, in which as much gum as possible is dissolved. The solution must be filtered, and is very troublesome to prepare. It is applied in the same way as glycerine, but the gum hardens at the edge of the cover-glass, so that the cement can be applied after a few days without risk, and there is no danger of leakage. While the gum is hardening it is well to make slight pressure on the cover-glass, so as to keep it in close apposition with the slide. This may be done by a small weight, as a rifle bullet, or by a compressorium, or mounting clip, which holds the glass down by a spring.

In some cases, instead of glycerine, a saturated solution of **acetate of potash** is employed. This is good for osmic acid preparations, which sometimes become diffusely black in glycerine, and for preparations stained in aniline violet, which soon lose their colour in glycerine.

Objects to be mounted in **Canada balsam** must usually be stained, since they become so transparent that, if unstained, they are invisible.

The balsam must be heated on a sand bath or in an oven until it becomes, on cooling, hard and brittle. Such hard balsam should be kept in quantity.

Hard tissues are sometimes mounted dry in this balsam directly, as was described in the section on bone; but for general mounting the balsam is dissolved in chloroform, benzole, or xylol, and filtered. The solution may be of any desired consistence. It should be kept in a stoppered bottle, from whose stopper a glass rod projects nearly to the bottom of the bottle. By this rod a drop of the solution can readily be removed.

A section to be mounted must be stained, washed in water, and then carefully removed on a lifter to a large watch-glass containing strong spirit. Before placing it in the spirit, the lifter should be touched with filter-paper, so as to remove all adhering water. While the object is in the spirit the watch-glass should be covered with a glass plate. If the section is thin and the spirit strong, the dehydration is effected in a few minutes, but it is better to leave the object in the spirit for at least ten minutes. It is then removed on the lifter, which is again touched with filter-paper, to remove adhering spirit, and the section is floated off into oil of cloves, contained in another watch-glass. If the section has been fully dehydrated, it floats to the surface of the oil of cloves, and in a few seconds is perfectly transparent, as can be seen by placing the watch-glass over some writing, when it will be found that the letters can be distinctly read through the transparent section. The section is then transferred on the lifter to the slide. Great care is to be taken to see that it lies flat, and is not folded on itself or wrinkled. The excess of oil of cloves is removed with filter-paper, but the section need not be dried, as Canada balsam mixes with oil of cloves. A drop of the balsam solution is then applied to the under surface of the cover-glass, and this is placed cautiously on the object, without including air bubbles. The preparation is then put away until the balsam hardens, during which time a slight pressure should be maintained on the cover-glass. The balsam soon sets about the edge of the preparation, but is very slow to harden in the centre. Hence, for some time, care must be taken not to handle the slide too roughly. For the same reason, microscopic preparations should always be kept lying flat, and never placed on their edges. Balsam preparations do not need to be cemented.

If the section, when transferred from spirit to oil of cloves, does not float, but sinks to the bottom and remains opaque, the dehydration has not been completed. The section must be again put back into spirit, and moved about until the adherent oil of cloves is washed away; then transferred to fresh spirit, in which it must remain until all the water is removed, and only then passed through oil of cloves and mounted in balsam. If the section is very delicate, so that there is danger of its being injured by being transferred from one watch-glass to another, it may be at once placed on the slide, in a drop of the staining fluid. The slide is placed in the moist chamber until the staining is accomplished. The dye is then washed away with a few drops of water, and absolute alcohol is dropped over the section for a few minutes. A drop of oil of cloves is then allowed to run under the section, which, when transparent, is mounted in the usual way. This method, in which all the steps of the process are carried out as the object lies on the slide, is more difficult than that described above.

Instead of oil of cloves, oil of bergamot, oil of cedar, and several other essential oils, may be employed; also a mixture of four parts of oil of turpentine and one of creasote.

Instead of Canada balsam, **dammar varnish** may be used. This is made as follows:—

Gum dammar, ½ oz.
Gum mastic, ½ oz.
Pure benzole, 3 fl. oz.
Dissolve and filter.

It need scarcely be said, that *objects which are to be mounted in glycerine or Farrant's solution do not require to be dehydrated, and should never be put into oil of cloves.*

If objects are very thick, they may require to be mounted in a cell. This is formed of a ring of glass, cemented to the slide by marine glue. The cell is filled with the mounting fluid, the cover-glass applied, and sealed on to the top of the cell with cement.

A good medium in which to mount objects that have to be put up in cells is **glycerine jelly.** This may be bought, and must be heated before use.

XII.—SUMMARY OF RE-AGENTS, &c.

I.—WATER.

Dissolves out colouring matter of blood corpuscles; raises sarcolemma from muscular fibres; causes the myeline sheath of nerve fibres to swell and leave the neurilemma.

II.—ACIDS.

1. **Nitric acid.**—20 per cent. for isolating smooth muscular fibres. With chlorate of potash, for isolating striped mus-

cular fibres. With chromic acid, for decalcifying bone and teeth. 5 per cent. solution as hardening agent.

2. **Sulphuric acid.**—For separating cells of hairs and nails.

3. **Acetic acid.**—Glacial, for preparing hæmin crystals; for demonstrating cells of tendons, &c., stained with picrocarmine. Dilute, $\frac{1}{2}$ to 1 per cent., for bringing out nuclei, and rendering white fibrous tissue transparent.

4. **Formic acid.**—1 per cent. in glycerine for mounting objects stained in picrocarmine. For reducing chloride of gold. See below.

5. **Tartaric acid.**—For reducing chloride of gold. See below.

6. **Tannic acid.**—One-half per cent. for separating hæmoglobin and stroma (œcoid and zooid) of red blood corpuscles.

7. **Boracic acid.**—2 per cent. for separating œcoid and zooid.

8. **Chromic acid.**
9. **Osmic acid.**
10. **Picric acid.**

} See HARDENING AGENTS.

III.—ALKALIES AND ALKALINE EARTHS.

1. **Potash.**—40 per cent. for isolating smooth muscular fibres and cells of myocardium. Dilute, 5–10 per cent., for clearing bundles of white fibrous tissue.

2. **Soda.**—2–4 per cent. for clearing bundles of white fibrous tissue.

3. **Ammonia.** — For dissolving neuro-keratin of nerve centres. See Exner's method for demonstrating medullated nerve fibres in cerebral cortex, p. 331.

4. **Baryta water.**
5. **Lime water.**

} For dissolving cementing substance of fibrous tissue, cartilage, &c.

IV.—SALTS.

1. **Chloride of sodium.**—In powder, for preparing hæmin crystals. In solution, 10 per cent., for showing exosmosis in red blood corpuscles; for dissolving cementing substances of fibrous tissue, &c. Dilute, $\frac{1}{2}$–$\frac{3}{4}$ per cent. (normal salt solution), as an indifferent fluid in which to examine all fresh tissues.

2. **Sulphate of soda.**—5 per cent. to dilute blood in counting corpuscles. See Müller's Fluid.

3. **Acetate of potash.**—Saturated solution, instead of glycerine, to mount objects stained in osmic acid or aniline violet.

4. **Bichromate of potash.**
5. **Bichromate of ammonia.**
6. **Neutral chromate of ammonia.**

} See section on Hardening.

7. **Chlorate of potash.**—With nitric acid, for isolating striped muscular fibres.

8. **Alum.**
9. **Borax.**

} Enter into composition of staining fluids.

10. **Nitrate of silver.**
11. **Chloride of gold.**

} See Staining Agents.

12. **Bichloride of mercury.**—Sometimes used in concentrated watery solution as a hardening reagent. Enters into **Hayem's solution** for preserving the third corpuscular element of the blood.

Corrosive sublimate, $\frac{1}{2}$.
Chloride of sodium, 1.
Sulphate of soda, 5.
Water, 100.

A large drop of this is placed on the finger. The skin is punctured with a fine needle through the drop, so as to bring the blood at once into contact with the preservative fluid, with which it is mixed with the point of the needle. A drop of the mixture shows for many hours the small discoid bodies (Blutplättchen of Bizzozero—Hæmatoblasts of Hayem) about one-third the diameter of the red corpuscles. This method is far superior to that by salt solution coloured with aniline violet, described on p. 33.

V.—ALCOHOLS.

1. **Ethylic alcohol.**—Absolute, for hardening tissues, dehydrating, &c. Dilute (rectified spirit, 1 vol.; water, 2 vols.), for macerating epithelia, &c.

Methylated spirit.—For ordinary hardening, &c.

2. **Glycerine.**—For mounting.

VI.—ETHER.

Dissolves fat, and fatty part of white substance of Schwann.

VII.—BENZOLE.

For dissolving Canada balsam, gum dammar, and gum mastic. **Xylol,** for demonstrating course of fibres in nerve centres (p. 330); for dissolving Canada balsam.

VIII.—ESSENTIAL OILS.

1. **Turpentine.**—For dissolving paraffin. Turpentine, 4, creasote, 1, for dissolving spermaceti and castor oil embedding mass.

2. **Oil of cloves.**—For clearing sections previous to mounting in balsam.

3. **Oil of bergamot.**—For clearing sections cemented to slide by collodion.

IX.—EMBEDDING MASSES.

1. **Wax and oil.**—Equal parts.
2. **Spermaceti,** 4; castor oil, 1.
3. **Paraffin.**
4. **Collodion,** or celloidin.
5. Egg **albumen.**

See section on Embedding (p. 355).

X.—MACERATING AGENTS.

See p. 48.

XI.—HARDENING AGENTS.

See p. 350.

XII.—MOUNTING FLUIDS.

1. **Glycerine.**

2. **Acetate of potash.**—Saturated solution, for objects stained in osmic acid or methyl violet.

3. **Farrant's solution.**

4. **Canada balsam.**—Heated until it becomes hard on cooling, for mounting bone and teeth. olved in benzole for general mounting.

5. **Dammar varnish.**

XIII.—STAINING FLUIDS.

Of the innumerable substances which have been used for staining microscopic objects, only the more important and generally useful will be given here.

1. **Carmine** (Gerlach). Carmine is insoluble in water, but dissolves readily in ammonia. The presence, however, of free ammonia in the solution causes the staining to be diffuse and unsatisfactory. It is necessary, therefore, to have a perfectly neutral carminate of ammonia, which is freely soluble in water.

One gram of carmine is dissolved in 1–2 cc. strong liquor ammoniæ, and diluted with 50–100 cc. water. The solution is left in an open bottle until it putrifies. After some weeks the putrefaction is ended, and the free ammonia has been completely dissipated. The solution is then boiled for a few minutes, filtered, and a few drops of a solution of carbolic acid, or of chloral (Hoyer), are added. Such a solution may be diluted with water when required for use. It stains nuclei most intensely, also protoplasm, connective-tissue fibres, axis cylinders of nerves, decalcified tissue of bone, and teeth. It is peculiarly useful for the central nervous system. The sections, after being stained, should be washed in water, and then placed for a short time in very dilute acetic acid, which fixes the colour. They may be then mounted in balsam, or in glycerine containing 1 per cent. formic acid.

It is not always easy to get a good carmine staining fluid—that is, one which has an elective power, and does not colour diffusely. Consequently, carminate of ammonia is not employed now so frequently as it formerly was. Numerous combinations of carmine have been devised, which give more or less satisfactory results. Of these, the most valuable are the following:—

2. **Picrocarmine.**—This is one of the most useful of staining substances. It was introduced by Schwartz, but popularised by Ranvier.

One gram of carmine is rubbed up with 30 drops of strong liquor ammoniæ, and diluted with 200 cc. water. To this 100 cc. of a cold saturated solution of picric acid are added, with constant stirring. The mixture is slowly evaporated on a water bath to two-thirds its original volume, and filtered when cold. A few drops of carbolic acid, or of a solution of chloral, are added, to make it keep.

Picrocarmine may be bought from the druggists in powder and in solution, but, in our experience, it is always worthless. According to Weigert, a bad picrocarmine may be improved by adding to its solution a trace of acetic acid, and if any precipitate occurs dissolving it by a little ammonia. We have not found this statement confirmed. The best picrocarmine is to be had from the *garçon* of M. Ranvier's laboratory in the College of France. It is far superior to any other which we have seen. In its manufacture the evaporation is effected at the temperature of the air, and occupies many weeks. When solid picrocarmine is employed it should be dissolved in water in the proportion of 1 per cent.

Picrocarmine gives a double staining. Nuclei, connective-tissue fibres, decalcified bone, axis cylinders are stained red; protoplasm and muscle, orange; while horny and elastic tissues are stained bright yellow. The staining is effected quickly. The sections after staining must not be left too long in water or alcohol, which dissolve out the picric acid, and remove the yellow colour. They may be mounted in balsam, but better in glycerine containing 1 per cent. formic acid, which fluid fixes the red colour, and increases the election of the dye.

3. **Borax carmine** (Grenacher).—Borax, 2.0; carmine, 0.5; distilled water, 100; mixed in a capsule, and heated to boiling. To the solution, which has a dark purple colour, 5 per cent. acetic acid is added, drop by drop, until the colour changes to red. The mixture is allowed to stand 24 hours, filtered, and some carbolic acid added.

This stains sections very rapidly, but the staining is quite diffuse. In order to remedy this, the section, on removal from the carmine fluid, is placed for a few minutes in 70 per cent. alcohol, containing 1 per cent. hydrochloric acid. This removes the colour from all parts except the nuclei, which remain of an intense red. The section is then washed in water, and mounted in glycerine or balsam.

An alcoholic solution of borax carmine has also been prepared by Grenacher, and is highly spoken of. In our experience it gives less satisfactory results than that just described.

4. **Alum carmine** (Grenacher).—1 gram of carmine is boiled for 20 minutes in 100 cc. of a 5 per cent. solution of alum, and filtered when cold. It stains nuclei of a purple-red colour. In our opinion it is inferior to the following:—

5. **Alum cochineal** (Czoker).—7 grams of cochineal are rubbed up in a mortar with 7 grams of burnt alum. The powder is placed in a flask, and 700 cc. water added. It is boiled until the volume is reduced to 400 cc., filtered when cool, and some carbolic acid added. This is an extremely useful staining fluid, particularly for class purposes, as sections are stained quickly by it, but may be left in it an indefinite time without risk of being over-stained. It is elective, and generally permanent. Nuclei and axis cylinders are stained of a bluish-

red colour; protoplasm and connective tissue only faintly; horny substances often have a yellowish tinge. Sections may be mounted in glycerine or balsam.

A combination of carmine with lithia has been recently recommended by Orth. We have no experience of it.

6. **Logwood** (Böhmer).—This is probably the most useful of all dyes. It stains nuclei of a blue colour, and with a sharpness and clearness which is scarcely equalled by any other staining re-agent. It has only one disadvantage: it frequently fades after a time. It may be made in various ways, from hæmatoxylin, extract of logwood, or infusion of the chips. In all cases the essential constituents are logwood and alum. The following method is that which we generally follow. It gives most satisfactory results:—

One part of logwood chips is infused for 6–10 hours in a warm place in 10 times its weight of water. The infusion is filtered, and to it is slowly added $\frac{1}{2}$ per cent. solution of alum in water, until the brown colour changes to a rich purple. Of the alum solution there will be required about three times the volume of the original infusion. The mixture is left in an open vessel for some days, when the colour will become much more intense. A little alcohol is added, to prevent putrefaction (Rutherford).

This solution can be used undiluted. As it is always turbid, it must be filtered immediately before use. It stains in a few minutes, and the sections must not be left in it too long. A little experience will enable the progress of the staining to be judged of, for which purpose the sections may be removed from time to time, and placed in water. Sections of objects which have been hardened in chromic acid or its salts stain more readily if they are placed for a few minutes in 1 per cent. bicarbonate of soda, and then washed in water before being put into the logwood. Logwood preparations may be mounted in glycerine or balsam. When balsam is used many fine details are lost, in consequence of the great transparency of all the unstained parts. This may be remedied by a double staining; that is, the sections are first stained in some dye which colours somewhat diffusely, and then in logwood, which stains the nuclei. Carmine or picrocarmine are good stains to combine in this way with logwood; but the best and most generally useful double stain is that got by logwood and eosine.

7. **Eosine** (E. Fischer).—The potash salt of tetrabromide of fluorescine is a red crystalline powder. It is very soluble in water and spirit, the solution being strongly fluorescent. It may be at once dissolved, or it may be previously decomposed as follows:—A watery solution is made, and to this dilute hydrochloric acid is added, drop by drop, until all the colouring matter precipitates. The precipitate is collected on a filter, and washed with distilled water. After all the acid has been removed, the colouring matter commences to become soluble in the water, and may be dissolved in it. But it is much more soluble in alcohol, in which it may be dissolved by simply pouring some spirit on the filter after the acid has been washed away by water. Eosine stains very intensely and diffusely. There are, however, certain objects for which it has a special affinity. It stains red blood corpuscles a salmon-red colour (Wissozky). Elastic and horny tissues are coloured a bright rose-red. The parietal cells of the stomach glands are stained bright red, as is muscle, both striped and smooth.

Certain cells have, contained in their protoplasm, granules which have a peculiar power of staining with eosine. Such cells occur in the tissues and in small numbers in the blood in health, but they are much more numerous in the blood in leucocythæmia. They have been studied specially by Ehrlich, who calls them eosinophilous cells. To stain blood with eosine, a thin layer of blood is smeared on a cover-glass, and rapidly dried by blowing a current of air over it. The cover-glass is then passed two or three times through the flame of a small Bunsen burner, so as to heat the layer of blood, and make its albuminous constituents insoluble. When the glass has cooled there is placed on it some drops of a solution of eosine in glycerine. After lying for a few hours this is washed off with water, the glass allowed to dry, a drop of balsam applied, and the cover-glass placed on the slide. The eosinophilous cells will be evident from the intensely red colour of their granules.

Eosine is, however, seldom used alone, but usually in conjunction with some elective stain, of which logwood is the best. It has been proposed to dissolve eosine in the logwood solution, and effect the double staining at one operation, but it is better to stain first in logwood, then in an alcoholic solution of eosine, which stains and dehydrates at the same time. The strength of the eosine solution is not of much consequence, as any excess of colour can be readily removed by spirit. When the desired tint has been obtained, the sections may be passed rapidly through oil of cloves and mounted in balsam, or may be washed in water and mounted in glycerine. This double staining, which is of very general applicability, gives peculiarly beautiful results where muscular and glandular tissues occur together in the same section, as, for example, in the prostate, tongue, stomach, intestine, &c. In reticular cartilage the appearances are very distinct, as logwood stains the hyaline intercellular substance, while the cells and elastic reticulum are intensely stained by the eosine.

A combination of eosine and picric acid is often used.

8. **Purpurine** (Ranvier).—This substance, which is not of any general utility, is useful for staining hyaline cartilage. 200 cc. of a ½ per cent. solution of alum are boiled in a capsule, and, while boiling, some purpurine in powder is added; the heat is continued so long as any purpurine dissolves: the fluid is then filtered hot into a bottle containing 60 cc. of rectified spirit. The solution is of a red colour and strongly fluorescent. It requires twenty-four to forty-eight hours to stain. It is an elective dye, colouring chiefly the nuclei. It has one advantage; it stains objects which have been in chromic acid as well as those which have been hardened in other fluids.

Of the numerous aniline dyes, a great number have been employed in histology. It is more particularly in pathological investigations that these reagents are of use, and much of our knowledge of parasitic micro-organisms is due to their employment.

Of the many aniline dyes which have been employed in normal histology, few are of any real utility. The more important of them are—

9. **Aniline blue-black.**—This is a very useful dye, particularly in the study of the nervous centres. It stains axis cylinders and nerve cells a bluish-grey colour, which is very agreeable to the eye, and which is fairly permanent. It was first employed by Sankey in his work on the cerebellum. It may be used in watery solution 1–5 per cent. It stains in a few minutes, and any excess of colour is readily removed by washing in spirit. A more ready way is to use an alcoholic solution one per thousand (p. 330). This stains and dehydrates at the same time. The stained sections are rapidly washed in spirit, passed through oil of cloves, and mounted in balsam. A double staining with logwood and aniline black gives good results, particularly for the cerebellum: the logwood stains the granule layer, the aniline the cells of Purkinje. The two dyes may be mixed together, or the sections may be stained first in logwood, and then in the aniline.

10. **Soluble aniline blue.**—1 per cent. solution in water. Useful for nerve-centres. Stains axis cylinders and nerve cells. Sections should be mounted in balsam. Stains also the parietal cells of the gastric glands (Heidenhain). Double staining with cochineal and aniline blue gives good results in the case of the stomach. The nuclei are red; the chief cells a fainter red; and the parietal cells blue: the blue colour, however, soon fades.

11. **Acid fuchsin.**—Used by Weigert for staining the medullated nerve fibres in the brain and spinal cord: see p. 331.

The remaining aniline dyes resemble each other closely in their action. A section immersed in a watery solution of one of them is soon deeply and uniformly stained. It is then placed in spirit, which rapidly removes the colour from nearly all parts except the nuclei, which are thus sharply and clearly defined (Hermann). The nuclei of the lymph corpuscles and connective-tissue cells are more deeply stained than are those of epithelium. After removal from the spirit, the sections may be cleared in oil of cloves and mounted in balsam. In glycerine the colour soon fades.

12. **Basic** or **ordinary Fuchsin,** or magenta.—Some of the crystals are dissolved in spirit, and the solution diluted with water. It is very useful for staining fresh tissues, as epithelial cells and blood corpuscles. This dye is now largely used in pathological investigation as a stain for the bacillus of tubercle (Koch).

13. **Methyl violet** (Jürgens).—In 1 per cent. watery solution or more dilute. Stains nuclei. Very useful for the skin: stains the superficial layer of the stratum corneum, and the stratum lucidum of the epidermis, and the internal root-sheath of the hairs (Unna). A double staining with picrocarmine and aniline violet gives very beautiful and instructive preparations. Mounted in balsam, the colour holds often for years. It holds also in acetate of potash, but soon fades in glycerine. In pathology, methyl violet is much used in the study of amyloid degeneration. It stains the normal tissues blue; the amyloid parts red.

14. **Gentian violet** and **Dahlia** resemble methyl violet closely in their colour and staining properties.

Dahlia (Zuppinger) has been much used for staining peculiar, very coarsely-granular cells, which are found in the connective tissue of different parts, chiefly in the neighbourhood of blood-vessels. They have been particularly studied by Ehrlich and Westphal, who call them *Mastzellen*. It would seem that they belong to the group of cells named by Waldeyer plasma cells, although this is denied by Westphal. The tissue must be hardened in alcohol, and deeply stained in an alcoholic solution of Dahlia strongly acidified with acetic acid. The excess of colour is removed by alcohol, and the preparation mounted in balsam. The granules in the cells are stained, while the nucleus does not take the colour.

15. **Iodine green.**—1–5 per cent. watery solution. Stains nuclei, particularly of lymph corpuscles; also the cells of mucous glands. A double staining with picrocarmine and iodine green (Stirling) gives very beautiful results at first. The green soon fades.

16. **Safranin.**—Very useful for staining nuclei, particularly the intranuclear reticulum (Flemming. Pfitzner). Shows admirably the changes which occur in the reticulum during karyokinesis. Preparations should be hardened in $\frac{1}{4}$ per cent. chromic acid. Stained in safranin, 1; alcohol, 100; water, 300. Excess of colour removed by alcohol. The preparations must be passed rapidly through oil of cloves (since this extracts the colour), and mounted in balsam.

17. **Bismarck brown** (Weigert).—In 1 per cent. watery solution, stains nuclei with great sharpness. Is a very agreeable colour, and holds better than many aniline dyes.

18. **Nitrate of silver** (Recklinghausen).—To the employment of this substance is due a great part of our present knowledge of endothelium, and of the structure of blood-vessels and lymphatics, as well as many important facts concerning the connective tissues. The part to be stained with silver must be fresh. The silver is applied either by rubbing the part with the solid crystal, or by bringing it into contact with a solution of the salt. The strength of the solution is usually from $\frac{1}{4}$–$\frac{1}{2}$ per cent. Silver has very slight power of penetration, consequently the staining is usually superficial. Great care must be taken to have the surface on which the silver acts perfectly clean, and free from blood or other albuminous fluid. A few minutes is sufficient to allow for the action of the silver. The part is then washed in distilled water, and exposed to the light in water, glycerine, or spirit. It soon becomes brown from reduction of the silver, and may then be examined. The reduction takes place chiefly in the cementing substances, and spares the cells. Hence, the outlines of the elements of cellular structures, as epithelium, endothelium, smooth muscular tissue, tissue of myocardium, &c., are marked out distinctly by brown or black lines. In connective tissues the intercellular substances are stained, leaving the cells clear on the brown ground. In this way the shape and arrangement of the cells in the cornea, cartilage, tendon, &c., may be readily demonstrated. If the part is overstained the cells themselves may have a brown colour. The nucleus is the last part to take the stain.

Sometimes, instead of the so-called negative staining just described, in which the cells appear colourless, a positive staining is got in which the reduction takes place exclusively in the cells, and the intercellular substance is uncoloured. This has been studied chiefly in the cornea. It may be got if the cornea is rubbed with nitrate of silver while the animal is living. After some days the animal is killed, and the cornea is removed and examined. The same result can be got if the cornea, after its removal, is treated with silver, and then placed for some days in distilled water, until it swells (Ranvier). A positive staining is always preceded by a negative, which changes into positive only after some time.

Other salts of silver have been employed, but they present no advantage over the nitrate.

19. **Gold.**—The introduction of the use of gold salts into histology is due to Cohnheim. Through it the best part of our knowledge of the terminations and distribution of non-medullated nerves has been gained, as well as important information regarding other tissues. The salts of gold which are used in histology are the chloride, and the double chlorides of gold and potassium, and of gold and sodium. There are no preparations clearer or more demonstrative than those stained with gold when the process has been successful; but unfortunately there is no method which is more uncertain, and fails more frequently, than gold staining; and as yet the conditions for success are but imperfectly understood, so that failures cannot be certainly guarded against.

Gold is reduced in protoplasmic bodies, such as the cells of cartilage, connective tissue, cornea, epithelium, but leaves the intercellular substance free. In this way it is complementary to the ordinary negative staining by silver. The nuclei of the cells in gold preparations are often unstained. The chief use of gold, however, is in the study of the nerves, particularly of the fine non-medullated fibrils, or naked axis cylinders. In successful cases these are stained a deep purple or black colour, and can be traced with a distinctness and certainty that it is impossible to attain by any other means. Many of the terminal organs of the nerves, as those in muscles, are most readily and fruitfully studied in gold preparations. Numerous modifications of the gold method have been employed. The following are the most generally useful :—

(*a*). **Cohnheim's method.**—The perfectly fresh tissue is immersed in a solution of chloride of gold in distilled water, of the strength of ½ per cent., until it is coloured throughout of a straw-yellow colour. It is then washed in distilled water, and exposed to the light in distilled water slightly acidulated with acetic acid, until it has assumed a dark-purple colour. It is then prepared by section or otherwise, and mounted in glycerine.

(*b*). **Henocque's method.**—The tissue, after removal from ½ per cent. chloride of gold and potassium, s placed for twenty-four hours in distilled water, then in a saturated solution of tartaric acid. The vessel containing this is plunged into water nearly at the boiling point. The reduction of the gold is completed in about twenty minutes, or less.

Klein also has used this method, with great success, in the study of the nerves of the cornea.

(*c*). **Böttcher's method.**—After removal from the gold solution, the tissue is placed in a stoppered bottle, containing a mixture of formic acid, 1; amylic alcohol, 1; and water, 100. The bottle is kept in the dark. The reduction is accomplished in about twenty-four hours. This method gives good results for the frog's cornea, so far as the cells are concerned, but does not stain the nerves well.

(*d*). **Löwit's method.**—The tissue is placed for a few minutes in equal parts of formic acid (specific grav. 1120) and water, then for fifteen to thirty minutes in chloride of gold, 1–1½ per cent., until it is stained yellow throughout. It is then placed for twenty-four hours in formic acid, diluted with three parts of water, and finally for twenty-four hours longer in undiluted formic acid. While in the gold, and subsequently, it must be kept in the dark.

The discovery of this method constituted a real advance, as, owing to the previous immersion in formic acid, the reduction of the gold is much facilitated. It is very useful for the nerves in muscle, skin, intestine, frog's bladder, &c.

(*e*). **Methods of Ranvier.**—Ranvier found that the formic acid, as employed by Löwit, exerted too violent an action on the fresh tissues, and caused an alteration of many of their finer structures. He consequently sought a substitute, which he discovered in freshly-expressed lemon juice. The fresh object is placed in this for from five to ten minutes, then rapidly washed in distilled water, and placed in 1 per cent. chloride of gold for twenty to thirty minutes; then washed again in water, and placed in very dilute acetic acid (2 drops in 50 cc. water), in which it is exposed to the light. The reduction is accomplished, according to the intensity of the light, in from one to three days. To prevent further reduction and diffuse staining of the tissues, the object should then be placed in spirit, in which it may be kept for some time without disadvantage. This method rarely fails when applied to the cornea of the rabbit or guinea-pig. Sections are mounted in glycerine. After treatment by lemon juice and gold, the object may be placed in formic acid diluted with three parts of water, and kept in the dark for twenty-four hours, when reduction will have been accomplished. This is the best method for muscle.

Good preparations of the nerves of muscle, of the heart, &c., may be got by the following method, also due to Ranvier:—Four parts of a 1 per cent. solution of chloride of gold and one part of formic acid are boiled together and allowed to cool. Into this the fresh object is brought directly, and left for twenty minutes; then washed, and placed for twenty-four hours in formic acid diluted with four parts of water.

(*f*). **Method of Bremer.**—The tissue is placed in formic acid, diluted with three parts of water, until it becomes transparent; then from fifteen to twenty minutes in 1 per cent. chloride of gold; then again in dilute formic acid for twenty-four hours, during which time it must be kept in the dark. Then it is placed for two or three weeks in glycerine containing 20 per cent. formic acid. This method is very useful for muscle. The prolonged stay in acid glycerine softens the connective tissue between the fibres, so that these separate almost of themselves when the final preparation is being made.

Many other modifications have been employed. Klein has got very perfect results by placing the tissue (cornea), after the action of the gold, in dilute glycerine, and keeping it in a warm place until the reduction is accomplished. Sertoli leaves the tissue (papilla foliata of horse's tongue) from twenty-four to forty-eight hours in $\frac{1}{4}$ per cent. chloride of gold. It is then washed, placed for one to two days in 2 per cent. bichromate of potash, then carefully washed and placed in absolute alcohol, to harden and complete the reduction. Gerlach has used very dilute solutions of chloride of gold—one part in 10,000—to stain the nerve fibrils in the grey substance of the spinal cord (a very difficult and uncertain method), and to demonstrate the terminations of nerves in muscle. Ranvier employs a solution of chloride of gold and potassium of the same strength, in the study of the developing blood-vessels in the omentum of the rabbit. The membrane is first treated with dilute alcohol for twenty-four hours; then washed and spread on a slide, and the gold solution dropped on it. After an hour it is again washed and mounted in glycerine. After some days' exposure to the light the reduction is accomplished. This method is easily carried out, and gives good results.

XIII.—ARTIFICIAL DIGESTION AS A HISTOLOGICAL METHOD.

Stirling was the first to submit a tissue to a digestive mixture, previous to its microscopic examination. Since then a great number of tissues and organs have been studied, after having undergone the action of either artificial gastric juice or pancreatic fluid. The results which have as yet been got are not devoid of interest, although they are, so far, somewhat discrepant.

The details of the employment of this method must vary in each case. The piece of tissue may be first digested and then cut into sections, or the sections may first be made, and then submitted to digestion. In this latter case the sections are mounted in a large drop of the digestive fluid, and the slide is placed in an oven at the temperature of 100° F. to 104° F., suitable precautions being taken to prevent evaporation of the fluid, and to maintain the temperature constant. The preparation can be removed and examined at intervals. It will then be seen in what order the different elements of the tissue are dissolved by the digestive mixture, and what parts are readily attacked, and what parts resist the solvent action.

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